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(54) Title: *CHLAMYDIA* PROTEIN, GENE SEQUENCE AND USES THEREOF

(57) Abstract

A high molecular weight ("HMW") protein of *Chlamydia*, the amino acid sequence thereof, and antibodies that specifically bind the HMW protein are disclosed as well as the nucleic acid sequence encoding the same. Also disclosed are prophylactic and therapeutic compositions, comprising the HMW protein, a fragment thereof, or an antibody that specifically binds the HMW protein or a portion thereof, or the nucleotide sequence encoding the HMW protein or a fragment thereof, including vaccines.

CHLAMYDIA PROTEIN, GENE SEQUENCE AND USES THEREOF

1.   FIELD OF THE INVENTION

The present invention generally relates to a high
5 molecular weight ("HMW") protein of *Chlamydia*, the amino acid
sequence thereof, and antibodies, including cytotoxic
antibodies, that specifically bind the HMW protein. The
invention further encompasses prophylactic and therapeutic
compositions comprising the HMW protein, a fragment thereof,
10 or an antibody that specifically binds the HMW protein or a
portion thereof or the nucleotide sequence encoding the HMW
protein or a fragment thereof, including vaccines. The
invention additionally provides methods of preventing,
treating or ameliorating disorders in mammals and birds
15 related to *Chlamydia* infections and for inducing immune
responses to *Chlamydia*. The invention further provides
isolated nucleotide sequences and degenerate sequences
encoding the HMW protein, vectors having said sequences, and
host cells containing said vectors. Diagnostic methods and
20 kits are also included.

2.   BACKGROUND OF THE INVENTION

Chlamydia are prevalent human pathogens causing
disorders such as sexually transmitted diseases, respiratory
25 diseases including pneumonia, neonatal conjunctivitis, and
blindness. *Chlamydia* are obligate intracellular bacteria
that infect the epithelial lining of the lung, conjunctivae
or genital tract.   The most common species of *Chlamydia*
include *Chlamydia trachomatis, Chlamydia psittaci, Chlamydia*
30 *pecorum and Chlamydia pneumoniae*. Recently, the newly
designated species of *Chlamydia*, *C. pneumoniae* (formerly *C.*
trachomatis TWAR), has been implicated as a major cause of
epidemic human pneumonitis and perhaps may play a role in
atherosclerosis.
35   There are currently 18 recognized *C. trachomatis*
serovars, causing trachoma and a broad spectrum of sexually
transmitted diseases: with the A, B and C serovars being most

frequently associated with trachoma, while the D-K serovars
are the most common cause of genital infections.

C. trachomatis is the major cause of sexually
transmitted disease in many industrialized countries,
5 including the United States. While the exact incidence of C.
trachomatis infection in the U.S. is not known, current
epidemiological studies indicate that more than 4 million
chlamydial infections occur each year, compared to an
estimated 2 million gonococcal infections. While all racial,
10 ethnic and socioeconomic groups are affected, the greatest
prevalence of chlamydial infections occur among young, 12 to
20 year-old, sexually active individuals. Most genitourinary
chlamydial infections are clinically asymptomatic. Prolonged
carriage in both men and women is common. As many as 25% of
15 men and 75% of women diagnosed as having chlamydial
infections have no overt signs of infection. As a
consequence, these asymptomatic individuals constitute a
large reservoir that can sustain transmission of the agent
within the community.
20   Far from being benign, serious disease can develop
from these infections including: urethritis, lymphogranuloma
venereum (LGV), cervicitis, and epididymitis in males.
Ascending infections from the endocervix commonly gives rise
to endometritis, pelvic inflammatory disease (PID) and
25 salpingitis which can cause tubal occlusion and lead
ultimately to infertility.

C. trachomatis infection of neonates results from
perinatal exposure to the mother's infected cervix. Nearly
70% of neonates born vaginally to mothers with chlamydial
30 cervicitis become infected during delivery. The mucus
membranes of the eye, oropharynx, urogenital tract and rectum
are the primary sites of infection. Chlamydial
conjunctivitis has become the most common form of ophthalmia
neonatorum. Approximately 20-30% of exposed infants develop
35 inclusion conjunctivitis within 14 days of delivery even
after receiving prophylaxis with either silver nitrate or
antibiotic ointment. C.trachomatis is also the leading cause

of infant pneumonia in the United States. Nearly 10-20% of
neonates delivered through an infected cervix will develop
chlamydial pneumonia and require some type of medical
intervention.

5   In developing countries, ocular infections of
C.trachomatis cause trachoma, a chronic follicular
conjunctivitis where repeated scar formation leads to
distortion of the eyelids and eventual loss of sight.
Trachoma is the world's leading cause of preventable
10 blindness. The World Health Organization estimates that over
500 million people worldwide, including about 150 million
children, currently suffer from active trachoma and over 6
million people have been blinded by this disease.

            In industrialized countries, the costs associated
15 with treating chlamydial infections are enormous. In the
U.S., the annual cost of treating these diseases was
estimated at $2.5-3 billion in 1992 and has been projected to
exceed $8 billion by the year 2000.

            One potential solution to this health crisis would
20 be an effective chlamydial vaccine. Several lines of
evidence suggest that developing an effective vaccine is
feasible.

            Studies in both humans and primates have shown that
short-term protective immunity to *C. trachomatis* can be
25 produced by vaccinating with whole *Chlamydia*. However,
protection was characterized as short lived, serovar
specific, and due to mucosal antibody. Additionally, in some
vaccinees disease was exacerbated when these individuals
became naturally infected with a serovar different from that
30 used for immunization. This adverse reaction was ultimately
demonstrated to be due to a delayed-type hypersensitivity
response. Thus, the need exists to develop a subunit-based
chlamydial vaccine capable of producing an efficacious but
nonsensitizing immune response. Such a subunit vaccine may
35 need to elicit both mucosal neutralizing secretory IgA
antibody and/or cellular immune response to be efficacious.

Subunit vaccine development efforts to date have
focused almost exclusively on the major outer membrane
protein (MOMP). MOMP is an integral membrane protein of
approximately 40 kDa in size and comprises up to about 60% of
5 the infectious elementary body (EB) membrane protein
(Caldwell, H.D., J.Kromhout, and L.Schachter. 1981. _Infect._
Immun., 31:1161-1176). MOMP imparts structural integrity to
the extracellular EB and is thought to function as a porin-
like molecule when the organism is growing intracellularly
10 and is metabolically active. With the exception of four
surface exposed variable domains (VDI-VDIV), MOMP is highly
conserved among all 18 serovars. MOMP is highly immunogenic
and can elicit a local neutralizing anti-Chlamydia antibody.
However, problems exists with this approach.

15   To date, most MOMP-specific neutralizing epitopes
that have been mapped are located within the VD regions and
thus give rise only to serovar-specific antibody. Attempts
to combine serovar-specific epitopes in various vaccine
vectors (e.g. poliovirus) to generate broadly cross-reactive
20 neutralizing antibodies have been only marginally successful
(Murdin, A.D., H. Su, D.S. Manning, M.H. Klein, M.J. Parnell,
and H.D. Caldwell. 1993. _Infect. Immun._, 61:4406-4414;
Murdin, A.D., H. Su, M.H. Klein, and H.D. Caldwell. 1995.
Infect. Immun., 63:1116-1121).

25   Two other major outer membrane proteins in _C._
trachomatis, the 60 kDa and 12 kDa cysteine-rich proteins, as
well as the surface-exposed lipopolysaccharide, are highly
immunogenic but, unlike MOMP, have not been shown to induce a
neutralizing antibody (Cerrone et al., 1991, _Infect. Immun._,
30 _59_:79-90). Therefore, there remains a need for a novel
subunit-based chlamydial vaccine.

3.   **SUMMARY OF THE INVENTION**

An object of the present invention is to provide an
35 isolated and substantially purified high molecular weight
protein of a _Chlamydia_ sp. ("HMW protein"), wherein the HMW
protein has an apparent molecular weight of about 105-115

kDa, as determined by SDS-PAGE, or a fragment or analogue
thereof. Preferably the HMW protein has substantially the
amino acid sequence of any of SEQ ID Nos.: 2, 15 and 16.
Preferred fragments of the HMW protein include SEQ ID Nos: 3,
5 17, and 25-37. As used herein, "substantially the sequence"
is intended to mean that the sequence is at least 80%, more
preferably at least 90% and most preferably at least 95%
identical to the referenced sequence. Preferably, the HMW
protein is an outer membrane protein. More preferably, the
10 outer membrane HMW protein is surface localized. Preferably,
the HMW protein has a heparin binding domain. Preferably,
the HMW Protein has a porin-like domain. It is intended that
all species of *Chlamydia* are included in this invention,
however preferred species include *Chlamydia trachomatis*,
15 *Chlamydia psittaci*, *Chlamydia percorum* and *Chlamydia*
pneumoniae. The substantially purified HMW protein is at
least 70 wt% pure, preferably at least about 90 wt% pure, and
may be in the form of an aqueous solution thereof.

    Also included in this invention are recombinant
20 forms of the HMW protein, wherein in transformed *E. coli*
cells, the expressed recombinant form of the HMW protein has
an apparent molecular weight of about 105-115 kDa, as
determined by SDS-PAGE, or a fragment or analogue thereof.
The term HMW-derived polypeptide is intended to include
25 fragments of the HMW protein; variants of wild-type HMW
protein or fragment thereof, containing one or more amino
acid deletions, insertions or substitutions; and chimeric
proteins comprising a heterologous polypeptide fused to the
C-terminal or N-terminal or internal segment of a whole or a
30 portion of the HMW protein.

    As used herein and in the claims, the term "HMW
protein" refers to a native purified or recombinant purified
high molecular weight protein of a species of *Chlamydia*
wherein the apparent molecular weight (as determined by SDS-
35 PAGE) is about 105-115 kDa. As used herein and in the
claims, the term "rHMW protein" refers to recombinant HMW
protein.

Another object of the present invention is to
provide an isolated substantially pure nucleic acid molecule
encoding a HMW protein or a fragment or an analogue thereof.
Preferred is the nucleic acid sequence wherein the encoded

5   HMW protein comprises the amino acid sequence of any of SEQ
ID Nos.: 2, 15 and 16, or a fragment thereof, particularly
SEQ ID Nos.: 3, 17, 25-37. Also included is an isolated
nucleic acid molecule comprising a DNA sequence of any of SEQ
ID Nos.: 1, 23-24 or a complementary sequence thereof; a

10   fragment of the HMW DNA sequence having the nucleic acid
sequence of any of SEQ ID Nos.: 4-14, 18-22 or the
complimentary sequence thereto; and a nucleic acid sequence
which hybridizes under stringent conditions to any one of the
sequences described above. The nucleic acid that hybridizes

15   under stringent condition preferably has a sequence identity
of about 70 % with any of the sequences identified above,
more preferably about 90 %.

The production and use of derivatives and analogues
of the HMW protein are within the scope of the present

20   invention. In a specific embodiment, the derivative or
analogue is functionally active, *i.e.*, capable of exhibiting
one or more functional activities associated with a full-
length, wild-type HMW protein. As one example, such
derivatives or analogues which have the desired

25   immunogenicity or antigenicity can be used, for example, in
immunoassays, for immunization, etc. A specific embodiment
relates to a HMW fragment that can be bound by an anti-HMW
antibody. Derivatives or analogues of HMW can be tested for
the desired activity by procedures known in the art.

30   In particular, HMW derivatives can be made by
altering HMW sequences by substitutions, additions or
deletions that provide for functionally equivalent molecules.
Due to the degeneracy of nucleotide coding sequences, other
DNA sequences which encode substantially the same amino acid

35   sequence as a HMW gene may be used in the practice of the
present invention. These include but are not limited to
nucleotide sequences comprising all or portions of genes

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which are altered by the substitution of different codons
that encode a functionally equivalent amino acid residue
within the sequence, thus producing a silent change.
Likewise, the HMW derivatives of the invention include, but

5 are not limited to, those containing, as a primary amino acid
sequence, all or part of the amino acid sequence of a HMW
protein including altered sequences in which functionally
equivalent amino acid residues are substituted for residues
within the sequence resulting in a silent change. For

10 example, one or more amino acid residues within the sequence
can be substituted by another amino acid of a similar
polarity which acts as a functional equivalent, resulting in
a silent alteration. Substitutes for an amino acid within
the sequence may be selected from other members of the class

15 to which the amino acid belongs. For example, the nonpolar
(hydrophobic) amino acids include alanine, leucine,
isoleucine, valine, proline, phenylalanine, tryptophan and
methionine. The polar neutral amino acids include glycine,
serine, threonine, cysteine, tyrosine, asparagine, and

20 glutamine. The positively charged (basic) amino acids
include arginine, lysine and histidine. The negatively
charged (acidic) amino acids include aspartic acid and
glutamic acid.

        In a specific embodiment of the invention, proteins

25 consisting of or comprising a fragment of a HMW protein
consisting of at least 6 (continuous) amino acids of the HMW
protein is provided. In other embodiments, the fragment
consists of at least 7 to 50 amino acids of the HMW protein.
In specific embodiments, such fragments are not larger than

30 35, 100 or 200 amino acids. Derivatives or analogues of HMW
include but are not limited to those molecules comprising
regions that are substantially homologous to HMW or fragments
thereof (e.g., in various embodiments, at least 60% or 70% or
80% or 90% or 95% identity over an amino acid sequence of

35 identical size or when compared to an aligned sequence in
which the alignment is done by a computer homology program
known in the art) or whose encoding nucleic acid is capable

of hybridizing to a coding HMW sequence, under stringent, moderately stringent, or nonstringent conditions.

By way of example and not limitation, useful computer homology programs include the following: Basic
5 Local Alignment Search Tool (BLAST) (www.ncbi.nlm.nih.gov) (Altschul et al., 1990, J. of Molec. Biol., 215:403-410, "The BLAST Algorithm; Altschul et al., 1997, Nuc. Acids Res. 25:3389-3402) a heuristic search algorithm tailored to searching for sequence similarity which ascribes significance
10 using the statistical methods of Karlin and Altschul 1990, Proc. Nat'l Acad. Sci. USA, 87:2264-68; 1993, Proc. Nat'l Acad. Sci. USA 90:5873-77. Five specific BLAST programs perform the following tasks:

1) The BLASTP program compares an amino acid query
15 sequence against a protein sequence database.

2) The BLASTN program compares a nucleotide query sequence against a nucleotide sequence database.

3) The BLASTX program compares the six-frame conceptual translation products of a nucleotide query
20 sequence (both strands) against a protein sequence database.

4) The TBLASTN program compares a protein query sequence against a nucleotide sequence database translated in all six reading frames (both strands).

5) The TBLASTX program compares the six-frame
25 translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database.

Smith-Waterman (database: European Bioinformatics Institute wwwz.ebi.ac.uk/bic_sw/) (Smith-Waterman, 1981, J. of Molec. Biol., 147:195-197) is a mathematically rigorous
30 algorithm for sequence alignments.

FASTA (see Pearson et al., 1988, Proc. Nat'l Acad. Sci. USA, 85:2444-2448) is a heuristic approximation to the Smith-Waterman algorithm. For a general discussion of the procedure and benefits of the BLAST, Smith-Waterman and FASTA
35 algorithms see Nicholas et al., 1998, "A Tutorial on Searching Sequence Databases and Sequence Scoring Methods" (www.psc.edu) and references cited therein.

The HMW derivatives and analogues of the invention can be produced by various methods known in the art. The manipulations which result in their production can occur at the gene or protein level. For example, the cloned HMW gene

5 sequence can be modified by any of numerous strategies known in the art (Sambrook *et al.*, 1989, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). The sequence can be cleaved at appropriate sites with restriction

10 endonuclease(s), followed by further enzymatic modification if desired, isolated, and ligated *in vitro*. In the production of the gene encoding a derivative or analogue of HMW, care should be taken to ensure that the modified gene remains within the same translational reading frame as HMW,

15 uninterrupted by translational stop signals, in the gene region where the desired HMW activity is encoded.

Additionally, the HMW-encoding nucleic acid sequence can be mutated *in vitro* or *in vivo*, to create and/or destroy translation, initiation, and/or termination

20 sequences, or to create variations in coding regions and/or form new restriction endonuclease sites or destroy preexisting ones, to facilitate further *in vitro* modification. Any technique for mutagenesis known in the art can be used, including but not limited to, chemical

25 mutagenesis, *in vitro* site-directed mutagenesis (Hutchinson, C., *et al.*, 1978, J. Biol. Chem 253:6551), use of TAB® linkers (Pharmacia), etc.

Manipulations of the HMW sequence may also be made at the protein level. Included within the scope of the

30 invention are HMW protein fragments or other derivatives or analogues which are differentially modified during or after translation, *e.g.*, by glycosylation, lipidation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to

35 an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to specific chemical

cleavage by cyanogen bromide, trypsin, chymotrypsin, papain,
V8 protease, NaBH$_4$; acetylation, formylation, oxidation,
reduction; metabolic synthesis in the presence of
tunicamycin; etc.

5   In addition, analogues and derivatives of HMW can
be chemically synthesized. For example, a peptide
corresponding to a portion of a HMW protein which comprises
the desired domain, or which mediates the desired activity *in
vitro*, can be synthesized by use of a peptide synthesizer.

10 Furthermore, if desired, nonclassical amino acids or chemical
amino acid analogues can be introduced as a substitution or
addition into the HMW sequence. Non-classical amino acids
include but are not limited to the D-isomers of the common
amino acids, α-amino isobutyric acid, 4-aminobutyric acid,

15 Abu, 2-amino butyric acid, γ-Abu, ε-Ahx, 6-amino hexanoic
acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid,
ornithine, norleucine, norvaline, hydroxyproline, sarcosine,
citrulline, cysteic acid, t-butylglycine, t-butylalanine,
phenylglycine, cyclohexylalanine, β-alanine, fluoro-amino

20 acids, designer amino acids such as β-methyl amino acids, Cα-
methyl amino acids, Nα-methyl amino acids, and amino acid
analogues in general. Furthermore, the amino acid can be D
(dextrorotary) or L (levorotary).

Another object of the invention is to provide a

25 recombinant expression vector adapted for transformation of a
host or for delivery of a HMW protein to a host comprising
the nucleic acid molecule of SEQ ID No.: 1, 23 or 24 or any
fragment thereof. Preferably, the recombinant expression
vector is adapted for transformation of a host and comprises

30 an expression means operatively coupled to the nucleic acid
molecule for expression by the host of said HMW protein or
the fragment or analogue thereof. More preferred is the
expression vector wherein the expression means includes a
nucleic acid portion encoding a leader sequence for secretion

35 from the host or an affinity domain coupled to either the N-
or C-terminus of the protein or the fragment or analogue
thereof.

A further aspect of the invention includes a
transformed host cell containing an expression vector
described above and the recombinant HMW protein or fragment
or analogue thereof producible by the transformed host cell.

Still a further aspect of the invention is directed
to a HMW protein recognizable by an antibody preparation that
specifically binds to a peptide having the amino acid
sequence of SEQ ID No. 2, 15-16 or a fragment or
conservatively substituted analogue thereof.

Antigenic and/or immunogenic compositions are
another aspect of the invention wherein the compositions
comprise at least one component selected from the following
group:

  a)   a HMW protein, wherein the molecular weight is
       about 105-115 kDa, as determined by SDS-PAGE,
       or a fragment or analogue thereof;

  b)   an isolated nucleic acid molecule encoding a
       HMW protein, or a fragment or analogue
       thereof;

  c)   an isolated nucleic acid molecule having the
       sequence of SEQ ID Nos. 1, 22, 23 or 24, the
       complimentary sequence thereto or a nucleic
       acid sequence which hybridizes under stringent
       conditions thereto or fragment thereof;

  d)   an isolated recombinant HMW protein, or
       fragment or analogue thereof, producible in a
       transformed host comprising an expression
       vector comprising a nucleic acid molecule as
       defined in b) or c) and expression means
       operatively coupled to the nucleic acid
       molecule for expression by the host of said
       HMW protein or the fragment or analogue
       thereof;

  e)   a recombinant vector comprising a nucleic acid
       encoding a HMW protein or fragment or analogue
       thereof;

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f)   a transformed cell comprising the vector of e)
        and optionally an adjuvant, and a
        pharmaceutically acceptable carrier or diluent
        therefor, said composition producing an immune

5   response when administered to a host.

Preferred adjuvants include cholera holotoxin or subunits, *E.
coli* heat labile holotoxin, subunits and mutant forms
thereof, alum, QS21, and MPL. Particularly, preferred are
alum, LTR192G, mLT and QS21.

10   Also included are methods for producing an immune
response in a mammal or a bird comprising administering to
said mammal, an effective amount of the antigenic or the
immunogenic composition described above.

        Another aspect of the invention is directed to

15 antisera raised against the antigenic or immunogenic
composition of the invention, and antibodies present in the
antisera that specifically bind a HMW protein or a fragment
or analogue thereof. Preferably the antibodies bind a HMW
protein having the amino acid sequence of SEQ ID Nos.: 2, 15-

20 16 or fragment or a conservatively substituted analogue
thereof. Also included are monoclonal antibodies that
specifically bind a HMW protein or a fragment or analogue
thereof.

        A further aspect of the invention includes

25 pharmaceutical and vaccine compositions comprising an
effective amount of at least one component selected from the
following group:

        a)   a HMW protein, wherein the isolated protein
                molecular weight is about 105-115 kDa, as

30   determined by SDS-PAGE, or a fragment or
                analogue thereof;

        b)   an isolated nucleic acid molecule encoding a
                HMW protein, or a fragment or analogue
                thereof;

35   c)   an isolated nucleic acid molecule having the
                sequence of SEQ ID Nos.: 1, 22, 23 or 24 the
                complimentary sequence thereto or a nucleic

acid sequence which hybridizes under stringent
conditions thereto or a fragment thereof;

d)   an isolated recombinant HMW protein, or
        fragment or analogue thereof producible in a
        transformed host comprising an expression
        vector comprising a nucleic acid molecule as
        defined in b) or c) and expression means
        operatively coupled to the nucleic acid
        molecule for expression by the host of said
        HMW protein of a *Chlamydia* species or the
        fragment or analogue thereof;

e)   a recombinant vector, comprising a nucleic
        acid encoding a HMW protein or fragment or
        analogue thereof;

f)   a transformed cell comprising the vector of
        e),

g)   antibodies that specifically bind the
        component of a), b), c), d) or e), and

a pharmaceutically acceptable carrier or diluent therefor.
Preferred are vaccine compositions which are effective at the
mucosal level.

    The invention also includes a diagnostic reagent
which may include any one or more of the above mentioned
aspects, such as the native HMW protein, the recombinant HMW
protein, the nucleic acid molecule, the immunogenic
composition, the antigenic composition, the antisera, the
antibodies, the vector comprising the nucleic acid, and the
transformed cell comprising the vector.

    Methods and diagnostic kits for detecting *Chlamydia*
or anti-*Chlamydia* antibodies in a test sample are also
included, wherein the methods comprise the steps of:

a)   contacting said sample with an antigenic
        composition comprising *Chlamydia* HMW protein
        or a fragment or analogue thereof or
        immunogenic composition or antibodies thereto
        to form *Chlamydia* antigen: anti-*Chlamydia*
        antibody immunocomplexes, and further,

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b)   detecting the presence of or measuring the
       amount of said immunocomplexes formed during
       step a) as an indication of the presence of
       said *Chlamydia* or anti-*Chlamydia* antibodies in
5   the test sample.

The diagnostic kits for detecting *Chlamydia* or antibodies
thereto comprise antibodies, or an antigenic or immunogenic
composition comprising *Chlamydia* HMW protein or a fragment or
analogue thereof, a container means for contacting said
10 antibodies or composition with a test sample suspected of
having anti-*Chlamydia* antibodies or *Chlamydia* and reagent
means for detecting or measuring *Chlamydia* antigen: anti-
Chlamydia antibody immunocomplexes formed between said
antigenic or immunogenic composition or said antibodies and
15 said test sample.

       A further aspect of the present invention provides
methods for determining the presence of nucleic acids
encoding a HMW protein or a fragment or analogue thereof in a
test sample, comprising the steps of:
20   a)   contacting the test sample with the nucleic
              acid molecule provided herein to produce
              duplexes comprising the nucleic acid molecule
              and any said nucleic acid molecule encoding
              the HMW protein in the test sample and
25   specifically hybridizable therewith; and
       b)   determining the production of duplexes.

       The present invention also provides a diagnostic
kit and reagents therefor, for determining the presence of
nucleic acid encoding a HMW protein or fragment or analogue
30 thereof in a sample, comprising:
       a)   the nucleic acid molecule as provided herein;
       b)   means for contacting the nucleic acid with the
              test sample to produce duplexes comprising the
              nucleic acid molecule and any said nucleic
35   acid molecule encoding the HMW protein in the
              test sample and specifically hybridizable
              therewith; and

c)   means for determining the production of
      duplexes.

Also included in this invention are methods of
preventing, treating or ameliorating disorders related to
5 *Chlamydia* in an animal including mammals and birds in need of
such treatment comprising administering an effective amount
of the pharmaceutical or vaccine composition of the
invention.   Preferred disorders include a *Chlamydia* bacterial
infection, trachoma, conjunctivitis, urethritis,
10 lymphogranuloma venereum (LGV), cervicitis, epididymitis, or
endometritis, pelvic inflammatory disease (PID), salpingitis,
tubal occlusion, infertility, cervical cancer, and
artherosclerosis.   Preferred vaccine or pharmaceutical
compositions include those formulated for *in vivo*
15 administration to a host to confer protection against disease
or treatment therefor caused by a species of *Chlamydia*. Also
preferred are compositions formulated as a microparticle,
capsule, liposome preparation or emulsion.

20 4.   **ABBREVIATIONS**

anti-HMW	=	HMW polypeptide antibody or antiserum
ATCC	=	American Type Culture Collection
immuno-reactive	=	capable of provoking a cellular or humoral immune response
kDa	=	kilodaltons
OG	=	n-octyl β-D-glucopyranoside or octyl glucoside
OMP	=	outer membrane protein
OMPs	=	outer membrane proteins
PBS	=	phosphate buffered saline
PAGE	=	polyacrylamide gel electrophoresis
polypeptide	=	a peptide of any length, preferably one having ten or more amino acid residues
SDS	=	sodium dodecylsulfate

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SDS-PAGE   =   sodium dodecylsulfate polyacrylamide
                         gel electrophoresis

Nucleotide or nucleic acid sequences defined herein
are represented by one-letter symbols for the bases as
5 follows:

A (adenine)

C (cytosine)

G (guanine)

T (thymine)

10 U (uracil)

M (A or C)

R (A or G)

W (A or T/U)

S (C or G)

15 Y (C or T/U)

K (G or T/U)

V (A or C or G; not T/U)

H (A or C or T/U; not G)

D (A or G or T/U; not C)

20 B (C or G or T/U; not A)

N (A or C or G or T/U) or (unknown)

Peptide and polypeptide sequences defined herein
are represented by one-letter symbols for amino acid residues
25 as follows:

A (alanine)

R (arginine)

N (asparagine)

D (aspartic acid)

30 C (cysteine)

Q (glutamine)

E (glutamic acid)

G (glycine)

H (histidine)

35 I (isoleucine)

L (leucine)

K (lysine)
M (methionine)
F (phenylalanine)
P (proline)
5 S (serine)
T (threonine)
W (tryptophan)
Y (tyrosine)
V (valine)
10 X (unknown)

      The present invention may be more fully understood
by reference to the following detailed description of the
invention, non-limiting examples of specific embodiments of
15 the invention and the appended figures.

   5.   **BRIEF DESCRIPTION OF THE FIGURES**

      Figure 1:   Western blot analysis of *C. trachomatis* L_2
20   elementary bodies (EBs).
                          Gradient purified EBs were solubilized in
                          standard Laemmli SDS-PAGE sample buffer
                          containing 2-mercaptoethanol, boiled for ~3
                          minutes and loaded onto a 4-12% Tris-glycine
25   gradient gel containing SDS and
                          electrophoresed at 100V. Immediately
                          following electrophoresis, proteins were
                          electroblotted onto PVDF membranes at 4°C for
                          ~2.5 hours at ~50V. The blocked membrane was
30   probed using a 1/5,000 dilution of anti-rHMWP'
                          antibody (K196) for 1.5 hours at room
                          temperature. Following washing, the membrane
                          was treated with a 1/5,000 dilution of a goat
                          anti-rabbit IgG antibody conjugated to HRP for
35   1 hour at room temperature. The blot was
                          developed using a standard TMB substrate
                          system.

Three immunoreactive bands detected in EBs and
RBs. Dot indicates HMW Protein of about 105-
115 kDa.

Figure 2.   Consensus Nucleic Acid Sequence encoding the
open reading frame of the HMW protein from *C.
trachomatis* LGV L_2.

Figure 3.   Deduced Amino Acid Sequence of the HMW protein
from the PCR open reading frame from *C.
trachomatis* LGV L_2.

Figure 4.   SDS-PAGE of partially purified recombinant HMW
protein from *C. trachomatis* LGVL$_2$ expressed in
E. coli. Counterstained and prestained SDS-
PAGE standards were used as molecular weight
markers. The positions of the molecular
weight markers in the gel are noted on the
left and right side of the figure by lines to
the molecular weights (kDa) of some of the
markers. See Text Example 10 for details.
Lane A: Mark 12 Wide Range Molecular Weight
Markers (Novex); myosin, 200 Kdal; B-
galactosidase, 116.3 Kdal; phosphorylase B,
97.4 Kdal; bovine serum albumin, 66.3 Kdal.
Lane B: *C. trachomatis* L2 recombinant HMWP.
Lane C: SeeBlue Prestained Molecular Weight
markers (Novex); myosin, 250 Kdal; bovine
serum albumin, 98 Kdal; glutamic
dehydrogenase, 64 Kdal.

Figure 5.   Map of plasmids pAH306, pAH310, pAH312, pAH316
and the PCR open reading frame.

Figure 6.   Predicted amino acid sequences, of HMW Protein
for *C. trachomatis* L_2, B, and F.
The *C. trachomatis* L2 sequence is given in the
top line and begins with the first residue of
the mature protein, E. Potential eucaryotic
N-glycosylation sequences are underlined. A
hydrophobic helical region flanked by proline-
rich segments and of suitable length to span

the lipid bilayer is underlined and enclosed
in brackets. Amino acid differences
identified in the B and F serovars are
designated below the L₂ HMWP protein sequence.

5 **Figure 7**   Indirect florescence antibody staining of *C.
                   trachomatis* N11 (serovar F) inclusion bodies
                   using anti-rHMWP' antibody.
                   Panel A: Post-immunization sera from rabbit
                   K196. *Chlamydia* inclusion bodies are stained
10   yellow.
                   Panel B: Pre-immunization sera from rabbit
                   K196.

6. DETAILED DESCRIPTION OF THE INVENTION

15   The term "antigens" and its related term
"antigenic" as used herein and in the claims refers to a
substance that binds specifically to an antibody or T-cell
receptor. Preferably said antigens are immunogenic.
           The term "immunogenic" as used herein and in the
20 claims refers to the ability to induce an immune response,
e.g., an antibody and/or a cellular immune response in a an
animal, preferably a mammal or a bird.
           The term "host" as used herein and in the claims
refers to either *in vivo* in an animal or *in vitro* in
25 mammalian cell cultures.
           An effective amount of the antigenic, immunogenic,
pharmaceutical, including, but not limited to vaccine,
composition of the invention should be administered, in which
"effective amount" is defined as an amount that is sufficient
30 to produce a desired prophylactic, therapeutic or
ameliorative response in a subject, including but not limited
to an immune response. The amount needed will vary depending
upon the immunogenicity of the HMW protein, fragment, nucleic
acid or derivative used, and the species and weight of the
35 subject to be administered, but may be ascertained using
standard techniques. The composition elicits an immune
response in a subject which produces antibodies, including

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anti-HMW protein antibodies and antibodies that are
opsonizing or bactericidal. In preferred, non-limiting,
embodiments of the invention, an effective amount of a
composition of the invention produces an elevation of
5 antibody titer to at least three times the antibody titer
prior to administration. In a preferred, specific, non-
limiting embodiment of the invention, approximately 0.01 to
2000 μg and preferably 0.1 to 500 μg are administered to a
host. Preferred are compositions additionally comprising an
10 adjuvant.

Immunogenic, antigenic, pharmaceutical and vaccine
compositions may be prepared as injectables, as liquid
solutions or emulsions. The HMW protein may be mixed with
one or more pharmaceutically acceptable excipient which is
15 compatible with the HMW protein. Such excipients may
include, water, saline, dextrose, glycerol, ethanol, and
combinations thereof.

Immunogenic, antigenic, pharmaceutical and vaccine
compositions may further contain one or more auxiliary
20 substance, such as wetting or emulsifying agents, pH
buffering agents, or adjuvants to enhance the effectiveness
thereof. Immunogenic, antigenic, pharmaceutical and vaccine
compositions may be administered parenterally, by injection,
subcutaneously or intramuscularly.

25   Alternatively, the immunogenic, antigenic,
pharmaceutical and vaccine compositions formed according to
the present invention, may be formulated and delivered in a
manner to evoke an immune response at mucosal surfaces.
Thus, the immunogenic, antigenic, pharmaceutical and vaccine
30 compositions may be administered to mucosal surfaces by, for
example, the nasal, oral (intragastric), ocular, branchiolar,
intravaginal or intrarectal routes. Alternatively, other
modes of administration including suppositories and oral
formulations may be desirable. For suppositories, binders
35 and carriers may include, for example, polyalkalene glycols
or triglycerides. Oral formulations may include normally
employed incipients such as, for example, pharmaceutical

grades of saccharine, cellulose and magnesium carbonate.
These compositions can take the form of solutions,
suspensions, tablets, pills, capsules, sustained release
formulations or powders and contain about 0.001 to 95% of the
5 HMW protein. The immunogenic, antigenic, pharmaceutical and
vaccine compositions are administered in a manner compatible
with the dosage formulation, and in such amount as will be
therapeutically effective, protective or immunogenic.

          Further, the immunogenic, antigenic, pharmaceutical
10 and vaccine compositions may be used in combination with or
conjugated to one or more targeting molecules for delivery to
specific cells of the immune system, such as the mucosal
surface. Some examples include but are not limited to
vitamin B12, bacterial toxins or fragments thereof,
15 monoclonal antibodies and other specific targeting lipids,
proteins, nucleic acids or carbohydrates.

          The quantity to be administered depends on the
subject to be treated, including, for example, the capacity
of the individual's immune system to synthesize antibodies,
20 and if needed, to produce a cell-mediated immune response.
Precise amounts of active ingredient required to be
administered depend on the judgment of the practitioner.
However, suitable dosage ranges are readily determinable by
one skilled in the art and may be of the order of 0.1 to 1000
25 micrograms of the HMW protein, fragment or analogue thereof.
Suitable regimes for initial administration and booster doses
are also variable, but may include an initial administration
followed by subsequent administrations. The dose may also
depend on the route(s) of administration and will vary
30 according to the size of the host.

          The concentration of the HMW protein in an
antigenic, immunogenic or pharmaceutical composition
according to the invention is in general about 0.001 to 95%.
A vaccine which contains antigenic material of only one
35 pathogen is a monovalent vaccine. Vaccines which contain
antigenic material of several pathogens are combined vaccines
and also belong to the present invention. Such combined

vaccines contain, for example, material from various
pathogens or from various strains of the same pathogen, or
from combinations of various pathogens.

The antigenic, immunogenic or pharmaceutical
5 preparations, including vaccines, may comprise as the
immunostimulating material a nucleotide vector comprising at
least a portion of the gene encoding the HMW protein, or the
at least a portion of the gene may be used directly for
immunization.

10   To efficiently induce humoral immune responses
(HIR) and cell-mediated immunity (CMI), immunogens are
typically emulsified in adjuvants. Immunogenicity can be
significantly improved if the immunogen is co-administered
with an adjuvant. Adjuvants may act by retaining the
15 immunogen locally near the site of administration to produce
a depot effect facilitating a slow, sustained release of
antigen to cells of the immune system. Adjuvants can also
attract cells of the immune system to an immunogen depot and
stimulate such cells to elicit immune responses.

20   Many adjuvants are toxic, inducing granulomas,
acute and chronic inflammations (Freund's complete adjuvant,
FCA), cytolysis (saponins and Pluronic polymers) and
pyrogenicity, arthritis and anterior uveitis (LPS and MDP).
Although FCA is an excellent adjuvant and widely used in
25 research, it is not licensed for use in human or veterinary
vaccines because of its toxicity.

Desirable characteristics of ideal adjuvants
include:

        (1)   lack of toxicity;
30   (2)   ability to stimulate a long-lasting immune
response;

        (3)   simplicity of manufacture and stability in
long-term storage;

        (4)   ability to elicit either CMI or HIR or both to
35 antigens administered by various routes, if required;

        (5)   synergy with other adjuvants;

(6)   capability of selectively interacting with populations of antigen presenting cells (APC);

(7)   ability to specifically elicit appropriate T_H1 or T_H2 cell-specific immune responses; and

5   (8)   ability to selectively increase appropriate antibody isotype levels (for example, IgA) against antigens.

Immunostimulatory agents or adjuvants have been used for many years to improve the host immune responses to, for example, vaccines. Intrinsic adjuvants, such as

10 lipopolysaccharides, normally are the components of the killed or attenuated bacteria used as vaccines. Extrinsic adjuvants are immunomodulators which are typically non-covalently linked to antigens and are formulated to enhance the host immune responses. Thus, adjuvants have been

15 identified that enhance the immune response to antigens delivered parenterally. Aluminum hydroxide and aluminum phosphate (collectively commonly referred to as alum) are routinely used as adjuvants in human and veterinary vaccines. The efficacy of alum in increasing antibody responses to

20 diphtheria and tetanus toxoids is well established and a HBsAg vaccine has been adjuvanted with alum.

Other extrinsic adjuvants may include saponins complexed to membrane protein antigens (immune stimulating complexes), pluronic polymers with mineral oil, killed

25 mycobacteria in mineral oil, Freund's complete adjuvant, bacterial products, such as muramyl dipeptide (MDP) and lipopolysaccharide (LPS), as well as lipid A, and liposomes.

International Patent Application, PCT/US95/09005 incorporated herein by reference describes mutated forms of

30 heat labile toxin of enterotoxigenic *E. coli* ("mLT"). U.S. Patent 5,057,540, incorporated herein by reference, describes the adjuvant, Qs21, an HPLC purified non-toxic fraction of a saponin from the bark of the South American tree Quiliaja saponaria molina 3D-MPL is described in great Britain Patent

35 2,220,211, and is incorporated herein by reference.

U.S. Patent No. 4,855,283 granted to Lockhoff et al on August 8, 1989 which is incorporated herein by reference,

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teaches glycolipid analogues including N-glycosylamides, N-glycosylureas and N-glycosylcarbamates, each of which is substituted in the sugar residue by an amino acid, as immuno-modulators or adjuvants. Lockhoff reported that N-
5 glycosphospholipids and glycoglycerolipids, are capable of eliciting strong immune responses in both herpes simplex virus vaccine and pseudorabies virus vaccine. Some glycolipids have been synthesized from long chain-alkylamines and fatty acids that are linked directly with the sugars
10 through the anomeric carbon atom, to mimic the functions of the naturally occurring lipid residues.

    U.S. Patent No. 4,258,029 granted to Moloney, incorporated herein by reference thereto, teaches that octadecyl tyrosine hydrochloride (OTH) functioned as an
15 adjuvant when complexed with tetanus toxoid and formalin inactivated type I, II and III poliomyelitis virus vaccine. Lipidation of synthetic peptides has also been used to increase their immunogenicity.

    Therefore, according to the invention, the
20 immunogenic, antigenic, pharmaceutical, including vaccine, compositions comprising a HMW protein, or a fragment or derivative thereof or a HMW encoding nucleic acid or fragment thereof or vector expressing the same, may further comprise an adjuvant, such as, but not limited to alum, mLT, QS21 and
25 all those listed above. Preferably, the adjuvant is selected from alum, LT, 3D-mPL, or Bacille Calmette-Guerine (BCG) and mutated or modified forms of the above, particularly mLT and LTR192G. The compositions of the present invention may also further comprise a suitable pharmaceutical carrier, including
30 but not limited to saline, bicarbonate, dextrose or other aqueous solution. Other suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences, Mack Publishing Company, a standard reference text in this field, which is incorporated herein by reference in its entirety.
35   Immunogenic, antigenic and pharmaceutical, including vaccine, compositions may be administered in a suitable, nontoxic pharmaceutical carrier, may be comprised

in microcapsules, and/or may be comprised in a sustained
release implant.

Immunogenic, antigenic and pharmaceutical,
including vaccine, compositions may desirably be administered
5 at several intervals in order to sustain antibody levels,
and/or may be used in conjunction with other bacteriocidal or
bacteriostatic methods.

As used herein and in the claims, "antibodies" of
the invention may be obtained by any conventional methods
10 known to those skilled in the art, such as but not limited to
the methods described in Antibodies A Laboratory Manual (E.
Harlow, D. Lane, Cold Spring Harbor Laboratory Press, 1989)
which is incorporated herein by reference in its entirety.
The term "antibodies" is intended to include all forms, such
15 as but not limited to polyclonal, monoclonal, purified IgG,
IgM, IgA and fragments thereof, including but not limited to
fragments such as Fv, single chain Fv (scFv), F(ab')$_2$, Fab
fragments (Harlow and Leon, 1988, Antibody, Cold Spring
Harbor); single chain antibodies (U.S. Patent No. 4,946,778)
20 chimeric or humanized antibodies (Morrison et al., 1984,
Proc. Nat'l Acad. Sci. USA 81:6851); Neuberger et al., 1984,
Nature 81:6851) and complementary determining regions (CDR),
(see Verhoeyen and Windust, in Molecular Immunology 2ed., by
B.D. Hames and D.M. Glover, IRL Press, Oxoford University
25 Press, 1996, at pp. 283-325), etc.

In general, an animal (a wide range of vertebrate
species can be used, the most common being mice, rats, guinea
pig, bovine, pig, hamsters, sheep, birds and rabbits) is
immunized with the HMW protein or nucleic acid sequence or
30 immunogenic fragment or derivative thereof of the present
invention in the absence or presence of an adjuvant or any
agent that enhances the immunogen's effectiveness and boosted
at regular intervals. The animal serum is assayed for the
presence of desired antibody by any convenient method. The
35 serum or blood of said animal can be used as the source of
polyclonal antibodies.

For monoclonal antibodies, animals are treated as
described above. When an acceptable antibody titre is
detected, the animal is euthanized and the spleen is asepti-
cally removed for fusion. The spleen cells are mixed with a
5 specifically selected immortal myeloma cell line, and the
mixture is then exposed to an agent, typically polyethylene
glycol or the like, which promotes the fusion of cells.
Under these circumstances fusion takes place in a random
selection and a fused cell mixture together with unfused
10 cells of each type is the resulting product. The myeloma
cell lines that are used for fusion are specifically chosen
such that, by the use of selection media, such as HAT:
hypoxanthine, aminopterin, and thymidine, the only cells to
persist in culture from the fusion mixture are those that are
15 hybrids between cells derived from the immunized donor and
the myeloma cells. After fusion, the cells are diluted and
cultured in the selective media. The culture media is
screened for the presence of antibody having desired
specificity towards the chosen antigen. Those cultures
20 containing the antibody of choice are cloned by limiting
dilution until it can be adduced that the cell culture is
single cell in origin.

Antigens, Immunogens and Immunoassays

25   The HMW protein or nucleic acid encoding same, and
fragments thereof are useful as an antigen or immunogen for
the generation of anti-HMW protein antibodies or as an
antigen in immunoassays including enzyme-linked immunosorbent
assays (ELISA), radioimmmunoassays (RIA) and other non-enzyme
30 linked antibody binding assays or procedures known in the art
for the detection of anti-bacterial, anti-*Chlamydia*, and
anti-HMW protein antibodies. In ELISA assays, the HMW
protein is immobilized onto a selected surface, for example,
a surface capable of binding proteins such as the wells of a
35 polystyrene microtiter plate. After washing to remove
incompletely absorbed HMW protein, a nonspecific protein
solution that is known to be antigenically neutral with

regard to the test sample may be bound to the selected surface. This allows for blocking of nonspecific absorption sites on the immobilizing surface and thus reduces the background caused by nonspecific bindings of antisera onto

5   the surface.

The immobilizing surface is then contacted with a sample, such as clinical or biological materials, to be tested in a manner conducive to immune complex (antigen/antibody) formation. This may include diluting the

10 sample with diluents, such as solutions of bovine gamma globulin (BGG) and/or phosphate buffered saline (PBS)/Tween. The sample is then allowed to incubate for from 2 to 4 hours, at temperatures such as of the order of about 20° to 37°C. Following incubation, the sample-contacted surface is washed

15 to remove non-immunocomplexed material. The washing procedure may include washing with a solution, such as PBS/Tween or a borate buffer. Following formation of specific immunocomplexes between the test sample and the bound HMW protein, and subsequent washing, the occurrence,

20 and even amount, of immunocomplex formation may be determined by subjecting the immunocomplex to a second antibody having specificity for the first antibody. If the test sample is of human origin, the second antibody is an antibody having specificity for human immunoglobulins and in general IgG.

25   To provide detecting means, the second antibody may have an associated activity such as an enzymatic activity that will generate, for example, a color development upon incubating with an appropriate chromogenic substrate. Detection may then be achieved by detecting color generation.

30 Quantification may then be achieved by measuring the degree of color generation using, for example, a visible spectrophotometer and comparing to an appropriate standard. Any other detecting means known to those skilled in the art are included.

35   Another embodiment includes diagnostic kits comprising all of the essential reagents required to perform a desired immunoassay according to the present invention.

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The diagnostic kit may be presented in a commercially
packaged form as a combination of one or more containers
holding the necessary reagents. Such a kit may comprise HMW
protein or nucleic acid encoding same or fragment thereof, a
5 monoclonal or polyclonal antibody of the present invention in
combination with several conventional kit components.
Conventional kit components will be readily apparent to those
skilled in the art and are disclosed in numerous
publications, including Antibodies A Laboratory Manual (E.
10 Harlow, D. Lane, Cold Spring Harbor Laboratory Press, 1989)
which is incorporated herein by reference in its entirety.
Conventional kit components may include such items as, for
example, microtitre plates, buffers to maintain the pH of the
assay mixture (such as, but not limited to Tris, HEPES,
15 etc.), conjugated second antibodies, such as peroxidase
conjugated anti-mouse IgG (or any anti-IgG to the animal from
which the first antibody was derived) and the like, and other
standard reagents.

20 Nucleic Acids and Uses Thereof

The nucleotide sequences of the present invention,
including DNA and RNA and comprising a sequence encoding the
HMW protein or a fragment or analogue thereof, may be
synthesized using methods known in the art, such as using
25 conventional chemical approaches or polymerase chain reaction
(PCR) amplification using convenient pairs of oligonucleotide
primers and ligase chain reaction using a battery of
contiguous oligonucleotides. The sequences also allow for
the identification and cloning of the HMW protein gene from
30 any species of Chlamydia, for instance for screening
chlamydial genomic libraries or expression libraries.

The nucleotide sequences encoding the HMW protein
of the present invention are useful for their ability to
selectively form duplex molecules with complementary
35 stretches of other protein genes. Depending on the
application, a variety of hybridization conditions may be
employed to achieve varying sequence identities. In specific

aspects, nucleic acids are provided which comprise a sequence
complementary to at least 10, 15, 25, 50, 100, 200 or 250
nucleotides of the HMW protein gene (Figure 2). In specific
embodiments, nucleic acids which hybridize to an HMW protein
5 nucleic acid (e.g. having sequence SEQ ID NO: 1, 23 or 24)
under annealing conditions of low, moderate or high
stringency conditions.

    For a high degree of selectivity, relatively
stringent conditions are used to form the duplexes, such as,
10 by way of example and not limitation, low salt and/or high
temperature conditions, such as provided by 0.02 M to 0.15 M
NaCl at temperatures of between about 50°C to 70°C. For some
applications, less stringent hybridization conditions are
required, by way of example and not limitation such a 0.15 M
15 to 0.9 M salt, at temperatures ranging from between about
20°C to 55°C. Hybridization conditions can also be rendered
more stringent by the addition of increasing amounts of
formamide, to destabilize the hybrid duplex. Thus,
particular hybridization conditions can be readily
20 manipulated, and will generally be a method of choice
depending on the desired results. By way of example and not
limitation, in general, convenient hybridization temperatures
in the presence of 50% formamide are: 42°C for a probe which
is 95 to 100% homologous to the target fragment, 37°C for 90
25 to 95% homology and 32°C for 70 to 90% homology.

    Low, moderate and high stringency conditions are
well known to those of skill in the art, and will vary
predictably depending on the base composition and length of
the particular nucleic acid sequence and on the specific
30 organism from which the nucleic acid sequence is derived.
For guidance regarding such conditions see, for example,
Sambrook et al., 1989, Molecular Cloning, A Laboratory
Manual, Second Edition, Cold Spring Harbor Press, N.Y., pp.
9.47-9.57; and Ausubel et al., 1989, Current Protocols in
35 Molecular Biology, Green Publishing Associates and Wiley
Interscience, N.Y. which is incorporate herein, by reference.

In the preparation of genomic libraries, DNA
fragments are generated, some of which will encode parts or
the whole of *Chlamydia* HMW protein. The DNA may be cleaved
at specific sites using various restriction enzymes.
5 Alternatively, one may use DNase in the presence of manganese
to fragment the DNA, or the DNA can be physically sheared, as
for example, by sonication. The DNA fragments can then be
separated according to size by standard techniques, including
but not limited to, agarose and polyacrylamide gel
10 electrophoresis, column chromatography and sucrose gradient
centrifugation. The DNA fragments can then be inserted into
suitable vectors, including but not limited to plasmids,
cosmids, bacteriophages lambda or T_4, bacmids and yeast
artificial chromosome (YAC). (See, for example, Sambrook et
15 al., 1989, Molecular Cloning, A Laboratory Manual, 2d Ed.,
Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New
York; Glover, D.M. (ed.), 1985, DNA Cloning: A Practical
Approach, MRL Press, Ltd., Oxford, U.K. Vol. I, II.) The
genomic library may be screened by nucleic acid hybridization
20 to labeled probe (Benton and Davis, 1977, Science 196:180;
Grunstein and Hogness, 1975, Proc. Natl. Acad. Sci. U.S.A.
72:3961).

The genomic libraries may be screened with labeled
degenerate oligonucleotide probes corresponding to the amino
25 acid sequence of any peptide of HMW protein using optimal
approaches well known in the art. In particular embodiments,
the screening probe is a degenerate oligonucleotide that
corresponds to the sequence of SEQ ID NO: 4. In another
embodiment, the screening probe may be a degenerate
30 oligonucleotide that corresponds to the sequence of SEQ ID
NO:5. In an additional embodiment, any one of the
oligonucleotides of SEQ ID NOs: 6-9, 12-14 and 18-21 are used
as the probe. In further embodiments, any one of the
sequences of SEQ ID NOs: 1, 10-11, 22-24 or any fragments
35 thereof, or any complement of the sequence or fragments may
be used as the probe. Any probe used preferably is 15
nucleotides or longer.

Clones in libraries with insert DNA encoding the
HMW protein or fragments thereof will hybridize to one or
more of the degenerate oligonucleotide probes. Hybridization
of such oligonucleotide probes to genomic libraries are
5 carried out using methods known in the art. For example,
hybridization with the two above-mentioned oligonucleotide
probes may be carried out in 2X SSC, 1.0% SDS at 50°C and
washed using the same conditions.

In yet another aspect, clones of nucleotide
10 sequences encoding a part or the entire HMW protein or HMW-
derived polypeptides may also be obtained by screening
Chlamydia expression libraries. For example, *Chlamydia* DNA
or *Chlamydia* cDNA generated from RNA is isolated and random
fragments are prepared and ligated into an expression vector
15 (e.g., a bacteriophage, plasmid, phagemid or cosmid) such
that the inserted sequence in the vector is capable of being
expressed by the host cell into which the vector is then
introduced. Various screening assays can then be used to
select for the expressed HMW protein or HMW-derived
20 polypeptides. In one embodiment, the various anti-HMW
antibodies of the invention can be used to identify the
desired clones using methods known in the art. See, for
example, Harlow and Lane, 1988, Antibodies: A Laboratory
Manual, Cold Spring Harbor Laboratory Press, Cold Spring
25 Harbor, NY, Appendix IV. Clones or plaques from the library
are brought into contact with the antibodies to identify
those clones that bind.

In an embodiment, colonies or plaques containing
DNA that encodes HMW protein or HMW-derived polypeptide could
30 be detected using DYNA Beads according to Olsvick et al.,
29th ICAAC, Houston, Tex. 1989, incorporated herein by
reference. Anti-HMW antibodies are crosslinked to tosylated
DYNA Beads M280, and these antibody-containing beads would
then be used to adsorb to colonies or plaques expressing HMW
35 protein or HMW-derived polypeptide. Colonies or plaques
expressing HMW protein or HMW-derived polypeptide is
identified as any of those that bind the beads.

Alternatively, the anti-HMW antibodies can be nonspecifically immobilized to a suitable support, such as silica or Celite™ resin. This material would then be used to adsorb to bacterial colonies expressing HMW protein or HMW-
5 derived polypeptide as described in the preceding paragraph.

In another aspect, PCR amplification may be used to produce substantially pure DNA encoding a part of or the whole of HMW protein from *Chlamydia* genomic DNA. Oligonucleotide primers, degenerate or otherwise,
10 corresponding to known HMW protein sequences can be used as primers. In particular embodiments, an oligonucleotide, degenerate or otherwise, encoding the peptide having an amino acid sequence of SEQ ID NO: 2, 3 or 15-17 or any portion thereof may be used as the 5' primer. For fragment examples,
15 a 5' primer may be made from any one of the nucleotide sequences of SEQ ID NO: 4-7, 10, 12, 22-24 or any portion thereof. Nucleotide sequences, degenerate or otherwise, that are reverse complements of SEQ ID NO: 11, 13 or 14 may be used as the 3' primer.
20   PCR can be carried out, *e.g.*, by use of a Perkin-Elmer Cetus thermal cycler and Taq polymerase (Gene Amp™). One can choose to synthesize several different degenerate primers, for use in the PCR reactions. It is also possible to vary the stringency of hybridization conditions used in
25 priming the PCR reactions, to allow for greater or lesser degrees of nucleotide sequence similarity between the degenerate primers and the corresponding sequences in *Chlamydia* DNA. After successful amplification of a segment of the sequence encoding HMW protein, that segment may be
30 molecularly cloned and sequenced, and utilized as a probe to isolate a complete genomic clone. This, in turn, will permit the determination of the gene's complete nucleotide sequence, the analysis of its expression, and the production of its protein product for functional analysis, as described *infra*.
35   In a clinical diagnostic embodiment, the nucleic acid sequences of the HMW protein genes of the present invention may be used in combination with an appropriate

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indicator means, such as a label, for determining hybridization. A wide variety of appropriate indicator means are known in the art, including radioactive, enzymatic or other ligands, such as avidin/biotin and digoxigenin-

5 labelling, which are capable of providing a detectable signal. In some diagnostic embodiments, an enzyme tag such as urease, alkaline phosphatase or peroxidase, instead of a radioactive tag may be used. In the case of enzyme tags, colorimetric indicator substrates are known which can be

10 employed to provide a means visible to the human eye or spectrophotometrically, to identify specific hybridization with samples containing HMW protein gene sequences.

The nucleic acid sequences of the HMW protein genes of the present invention are useful as hybridization probes

15 in solution hybridizations and in embodiments employing solid-phase procedures. In embodiments involving solid-phase procedures, the test DNA (or RNA) from samples, such as clinical samples, including exudates, body fluids (e.g., serum, amniotic fluid, middle ear effusion, sputum, semen,

20 urine, tears, mucus, bronchoalveolar lavage fluid) or even tissues, is absorbed or otherwise affixed to a selected matrix or surface. The fixed, single-stranded nucleic acid is then subjected to specific hybridization with selected probes comprising the nucleic acid sequences of the HMW

25 protein encoding genes or fragments or analogues thereof of the present invention under desired conditions. The selected conditions will depend on the particular circumstances based on the particular criteria required depending on, for example, the G+C contents, type of target nucleic acid,

30 source of nucleic acid, size of hybridization probe etc. Following washing of the hybridization surface so as to remove non-specifically bound probe molecules, specific hybridization is detected, or even quantified, by means of the label. It is preferred to select nucleic acid sequence

35 portions which are conserved among species of *Chlamydia*. The selected probe may be at least 15 bp and may be in the range of about 30 to 90 bp.

Expression of the HMW protein Gene

Plasmid vectors containing replicon and control
sequences which are derived from species compatible with the
host cell may be used for the expression of the genes
5 encoding the HMW protein or fragments thereof in expression
systems. Expression vectors contain all the necessary
elements for the transcription and translation of the
inserted protein coding sequence. The vector ordinarily
carries a replication site, as well as marking sequences
10 which are capable of providing phenotype selection in
transformed cells. For example, *E. coli* may be transformed
using pBR322 which contains genes for ampicillin and
tetracycline resistance cells. Other commercially available
vectors are useful, including but not limited to pZERO,
15 pTrc99A, pUC19, pUC18, pKK223-3, pEX1, pCAL, pET, pSPUTK,
pTrxFus, pFastBac, pThioHis, pTrcHis, pTrcHis2, and pLEx.
The plasmids or phage, must also contain, or be modified to
contain, promoters which can be used by the host cell for
expression of its own proteins.
20   In addition, phage vectors containing replicon and
control sequences that are compatible with the host can be
used as a transforming vector in connection with these hosts.
For example, the phage in lambda GEM™-11 may be utilized in
making recombinant phage vectors which can be used to
25 transform host cells, such as *E. coli* LE392.
Promoters commonly used in recombinant DNA
construction include the β-lactamase (penicillinase) and
lactose promoter systems and other microbial promoters, such
as the T7 promoter system as described in U.S. Patent No.
30 4,952,496. Details concerning the nucleotide sequences of
promoters are known, enabling a skilled worker to ligate them
functionally with genes. The particular promoter used will
generally be matter of choice depending upon the desired
results.
35   In accordance with this invention, it is preferred
to make the HMW protein by recombinant methods, particularly
when the naturally occurring HMW protein as isolated from a

culture of a species of *Chlamydia* may include trace amounts
of toxic materials or other contaminants. This problem can
be avoided by using recombinantly produced HMW protein in
heterologous systems which can be isolated from the host in a
5 manner to minimize contaminants in the isolated material.
Particularly desirable hosts for expression in this regard
include Gram positive bacteria which do not have LPS and are,
therefore endotoxin free. Such hosts include species of
Bacillus and may be particularly useful for the production of
10 non-pyrogenic rHMW protein, fragments or analogues thereof.

A variety of host-vector systems may be utilized to
express the protein-coding sequence. These include but are
not limited to mammalian cell systems infected with virus
(*e.g.*, vaccinia virus, adenovirus, etc.); insect cell systems
15 infected with virus (*e.g.*, baculovirus); microorganisms such
as yeast containing yeast vectors, or bacteria transformed
with bacteriophage DNA, plasmid DNA, or cosmid DNA. Hosts
that are appropriate for expression of the HMW protein genes,
fragments, analogues or variants thereof, may include *E.*
20 *coli*, *Bacillus* species, *Haemophilus*, fungi, yeast, such as
Saccharomyces pichia, *Bordetella*, or the baculovirus
expression system may be used. Preferably, the host cell is
a bacterium, and most preferably the bacterium is *E. coli*, *B.*
subtilis or *Salmonella*.

25   The expression elements of vectors vary in their
strengths and specificities. Depending on the host-vector
system utilized, any one of a number of suitable
transcription and translation elements may be used. In a
preferred embodiment, a chimeric protein comprising HMW
30 protein or HMW-derived polypeptide sequence and a pre and/or
pro sequence of the host cell is expressed. In other
preferred embodiments, a chimeric protein comprising HMW
protein or HMW-derived polypeptide sequence fused with, for
example, an affinity purification peptide, is expressed. In
35 further preferred embodiments, a chimeric protein comprising
HMW protein or HMW-derived polypeptide sequence and a useful
immunogenic peptide or protein is expressed. In preferred

embodiments, HMW-derived protein expressed contains a
sequence forming either an outer-surface epitope or the
receptor-binding domain of native HMW protein.

Any method known in the art for inserting DNA
5 fragments into a vector may be used to construct expression
vectors containing a chimeric gene consisting of appropriate
transcriptional/ translational control signals and the
protein coding sequences. These methods may include *in vitro*
recombinant DNA and synthetic techniques and *in vivo*
10 recombinants (genetic recombination). Expression of a
nucleic acid sequence encoding HMW protein or HMW-derived
polypeptide may be regulated by a second nucleic acid
sequence so that the inserted sequence is expressed in a host
transformed with the recombinant DNA molecule. For example,
15 expression of the inserted sequence may be controlled by any
promoter/enhancer element known in the art. Promoters which
may be used to control expression of inserted sequences
include, but are not limited to the SV40 early promoter
region (Bernoist and Chambon, 1981, Nature 290:304-310), the
20 promoter contained in the 3' long terminal repeat of Rous
sarcoma virus (Yamamoto et al., 1980, Cell 22:787-797), the
herpes thymidine kinase promoter (Wagner et al., 1981, Proc.
Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory
sequences of the metallothionein gene (Brinster et al., 1982,
25 Nature 296:39-42) for expression in animal cells; the
promoters of β-lactamase (Villa-Kamaroff et al., 1978, Proc.
Natl. Acad. Sci. U.S.A. 75:3727-3731), *tac* (DeBoer et al.,
1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25), P_L, or *trc* for
expression in bacterial cells (see also "Useful proteins from
30 recombinant bacteria" in Scientific American, 1980, 242:74-
94); the nopaline synthetase promoter region or the
cauliflower mosaic virus 35S RNA promoter (Gardner et al.,
1981, Nucl. Acids Res. 9:2871), and the promoter of the
photosynthetic enzyme ribulose biphosphate carboxylase
35 (Herrera-Estrella et al., 1984, Nature 310:115-120) for
expression implant cells; promoter elements from yeast or
other fungi such as the Gal4 promoter, the ADC (alcohol

dehydrogenase) promoter, PGK (phosphoglycerol kinase)
promoter, alkaline phosphatase promoter.

Expression vectors containing HMW protein or HMW-
derived polypeptide coding sequences can be identified by
5 three general approaches: (a) nucleic acid hybridization, (b)
presence or absence of "marker" gene functions, and (c)
expression of inserted sequences such as reactivity with
anti-HMW antibody. In the first approach, the presence of a
foreign gene inserted in an expression vector can be detected
10 by nucleic acid hybridization using probes comprising
sequences that are homologous to the inserted HMW protein or
HMW-derived polypeptide coding sequence. In the second
approach, the recombinant vector/host system can be
identified and selected based upon the presence or absence of
15 certain "marker" gene functions (e.g., thymidine kinase
activity, resistance to antibiotics, transformation
phenotype, occlusion body formation in baculovirus, etc.)
caused by the insertion of foreign genes in the vector. For
example, if the HMW protein or HMW-derived polypeptide coding
20 sequence is inserted within the marker gene sequence of the
vector, recombinants containing the insert can be identified
by the absence of the marker gene function. In the third
approach, recombinant expression vectors can be identified by
assaying the foreign gene product expressed by the
25 recombinant. Such assays can be based, for example, on the
physical or functional properties of HMW protein or HMW-
derived polypeptide in in vitro assay systems, e.g., binding
to an HMW ligand or receptor, or binding with anti-HMW
antibodies of the invention, or the ability of the host cell
30 to hemagglutinate or the ability of the cell extract to
interfere with hemagglutination by Chlamydia.

Once a particular recombinant DNA molecule is
identified and isolated, several methods known in the art may
be used to propagate it. Once a suitable host system and
35 growth conditions are established, recombinant expression
vectors can be propagated and prepared in quantity. As
explained above, the expression vectors which can be used

include, but are not limited to, the following vectors or
their derivatives: human or animal viruses such as vaccinia
virus or adenovirus; insect viruses such as baculovirus;
yeast vectors; bacteriophage vectors (e.g., lambda), and
5 plasmid and cosmid DNA vectors, to name but a few.

In addition, a host cell strain may be chosen which
modulates the expression of the inserted sequences, or
modifies and processes the gene product in the specific
fashion desired. Expression from certain promoters can be
10 elevated in the presence of certain inducers; thus,
expression of the genetically engineered HMW protein or HMW-
derived HMW may be controlled. Furthermore, different host
cells have characteristic and specific mechanisms for the
translational and post-translational processing and
15 modification of proteins. Appropriate cell lines or host
systems can be chosen to ensure the desired modification and
processing of the foreign protein expressed.

The proteins, polypeptides, peptides, antibodies
and nucleic acids of the invention are useful as reagents for
20 clinical or medical diagnosis of Chlamydia infections and for
scientific research on the properties of pathogenicity,
virulence, and infectivity of Chlamydia, as well as host
defense mechanisms. For example, DNA and RNA of the
invention can be used as probes to identify the presence of
25 Chlamydia in biological specimens by hybridization or PCR
amplification. The DNA and RNA can also be used to identify
other bacteria that might encode a polypeptide related to the
Chlamydia HMW protein. The proteins of the invention may be
used to prepare polyclonal and monoclonal antibodies that can
30 be used to further purify compositions containing the
proteins of the invention by affinity chromatography. The
proteins can also be used in standard immunoassays to screen
for the presence of antibodies to Chlamydia in a sample.

35 7.   **BIOLOGICAL DEPOSITS**

Certain plasmids that contain portions of the gene
having the open reading frame of the gene encoding the HMW

protein of *Chlamydia* that are described and referred to
herein have been deposited with the American Type Culture
Collection (ATCC) located at 10801 University Boulevard,
Manassas, Virginia 20110-2209, U.S.A., pursuant to the
5 Budapest Treaty and pursuant to 37 CFR 1.808 and prior to the
filing of this application. The identifications of the
respective portions of the genes present in these plasmids
are shown below.

Samples of the deposited materials will become
10 available to the public upon grant of a patent based upon
this United Stated patent application. The invention
described and claimed herein is not to be limited by the
scope of the plasmids deposited, since the deposited
embodiment is intended only as an illustration of the
15 invention. Any equivalent or similar plasmids that encode
similar or equivalent proteins or fragments or analogues
thereof as described in this application are within the scope
of the invention.

20 Plasmid	ATCC Accession No.	Date Deposited
pAH342	ATCC 985538	September 8, 1997

8. EXAMPLES

The above disclosure generally describes the
25 present invention. A more specific description is provided
below in the following examples. The examples are described
solely for the purpose of illustration and are not intended
to limit the scope of the invention. Changes in form and
substitution of equivalents are contemplated as circumstances
30 suggest or render expedient. Although specific terms have
been employed herein, such terms are intended in a
descriptive sense and not for purposes of limitation.

Methods of molecular genetics, protein biochemistry
and immunology used but not explicitly described in the
35 disclosure and examples are amply reported in the scientific
literature and are well within the ability of those skilled
in the art.

8.1. EXAMPLE 1:   ISOLATION AND PURIFICATION OF MATURE
CHLAMYDIA PROTEINS

        McCoy cells were cultured either in standard 225 cm^2
tissue culture flasks or in Bellco spinner flasks (Cytodex
5 microcarrier, Pharmacia) at 37°C in 5% CO_2 using DMEM media
supplemented with 10% *Chlamydia*-antibody free fetal bovine
serum, glucose and nonessential amino acids. *C. trachomatis*
L_2 elementary bodies (ATCC VR-902B) were prepared from lysates
of infected McCoy cells. Basically, McCoy cells infected
10 with *C. trachomatis* L_2 (LGV) were sonicated and cellular
debris was removed by centrifugation. The supernatant
containing chlamydial elementary bodies (EBs) was then
centrifuged and the pellet containing EBs was resuspended in
Hanks' balanced salts solution (HBSS). RNase/DNase solution
15 was added and incubated at 37°C for 1 hour with occasional
mixing. The EB containing solution was layered onto a
discontinuous density gradient (40%, 44% and 54%) of
Angiovist 370 (mixture of diatrizoate melgumine and
diatrizoate sodium, Berlex Laboratories, Wayne, NJ) and
20 ultracentrifuged for separation of the EBs on the gradient.
After centrifugation the EBs were harvested from the gradient
between the interface of the 44% and 54% Angiovist 370
layers. The EBs were washed in phosphate buffered saline and
resuspended in HBSS.
25   Purified EBs were sequentially extracted with 0.1%
OGP [high ionic strength] in HBSS to remove peripheral
surface proteins and held on ice. The same EB preparation
was then extracted with 1.0% OGP, 10 mM DTT, 1 mM PMSF, 10 mM
EDTA, in a 50 mM Tris pH 7.4 buffer. Extracts were dialyzed
30 (3500 MWCO) to remove detergent and other reagents and
concentrated by lyophilization. Protein containing extracts
were diluted in HBSS and passed over commercially available
heparin-sepharose columns (HiTrap Col., Pharmacia). After
samples were applied to the heparin column nonadhered
35 proteins were removed by washing with excess HBSS. Bound
proteins were batch eluted with PBS containing 2 M sodium
chloride. Eluents were dialyzed extensively to remove salt

and then lyophilized. The heparin-binding proteins were size
fractionated by SDS-PAGE and visualized by silver staining or
analyzed by Western blotting. Protein(s) of about 105-115
KDa present in moderate amounts were detected as shown in
5 Figure 1. The isoelectric point of the native HMW protein
was determined to be about 5.95.

        To obtain one N-terminal amino acid sequence,
sufficient quantities of the HMW protein ($\geq$ 5 μg) were
electroblotted onto a PVDF membrane (Applied Biosystems), and
10 stained with Coomassie blue. Immobilized HMW protein was
released from the membrane and treated in situ with low
levels of endopeptidase Lys-C, endopeptidase Arg-C and/or
endopeptidase Glu-C to fragment the native protein. The
resulting peptide fragments were purified by HPLC and their
15 N-terminal amino acid sequences determined using an ABI 430
Protein Sequenator and standard protein sequencing
methodologies. The N-terminal amino acid sequence is:

        E-I-M-V-P-Q-G-I-Y-D-G-E-T-L-T-V-S-F-X-Y
20

and is denoted SEQ ID No.: 3.

        When a composite PDB+SwissProt+PIR+GenPept database
(>145 K unique sequences) was searched with the HMW protein
N-terminal sequence (20 residues) using rigorous match
25 parameters, no precise homologies were found. Thus the HMW
protein is a novel chlamydial protein. Since this protein
was isolated under conditions that should release only
peripheral membrane proteins (e.g. Omp2), these data indicate
that the HMW protein is a surface-associated protein.
30

**8.2.   EXAMPLE 2:   PREPARATION OF ANTIBODIES TO WHOLE
CHLAMYDIA EBs**

        To aid in the characterization of the HMW protein,
hyperimmune rabbit antisera was raised against whole EBs from
35 C. trachomatis L$_2$. Each animal was given a total of three
immunizations of about 250 μg of Chlamydia EBs per injection
(beginning with complete Freund's adjuvant and followed with

incomplete Freund's adjuvant) at approximately 21 day
intervals. At each immunization, approximately half of the
material was administered intramuscularly (i.m.) and half was
injected intranodally. Fourteen days after the third
5 vaccination a fourth booster of about 100 μg of EBs was given
i.m. and the animals exsanguinated 7-10 days later. A titre
of 1:100,000 was obtained as determined by ELISA.

8.3. EXAMPLE 3: DETERMINATION OF POST-TRANSLATIONAL
10 MODIFICATIONS
        Recently, several *C. trachomatis* membrane-
associated proteins have been shown to be post-
translationally modified. The 18 kDa and 32 kDa cysteine-
rich EB proteins, which are lectin-binding proteins, have
15 been shown to carry specific carbohydrate moieties (Swanson,
A.F. and C.C. Kuo. 1990. Infect. Immun. 58:502-507).
Incorporation of radiolabelled palmitic acid has been used to
demonstrate that the about 27 kDa *C. trachomatis* Mip-like
protein is lipidated (Lundemose, A.G., D.A. Rouch, C.W. Penn,
20 and J.H. Pearce. 1993. J. Bacteriol. 175:3669-3671). Swanson
et al. have discovered that the MOMP from the L_2 serovar
contains N-acetylglucosamine and/or N-acetylgalactosamine and
these carbohydrate moieties mediate binding of MOMP to Hela
cell membranes.
25   To ascertain whether the HMW protein is
glycosylated, EBs are grown on McCoy cells in the presence of
tritiated galactose or glucosamine, subjected to heparin
affinity chromatography and the heparin binding proteins
analyzed by SDS-PAGE and autoradiography. Briefly, McCoy
30 cells are grown in T225 flasks under standard conditions
(DMEM + 10% FCS, 35 ml per flask, 10% CO_2) to about 90%
confluency and inoculated with sufficient EBs to achieve 90%-
100% infectivity. Following a 3 hour infection period at
37°C cycloheximide is added (1 μg/ml) to inhibit host cell
35 protein synthesis and the cultures reincubated for an
additional 4-6 hours. Approximately 0.5 mCi of tritiated
galactose (D-[4,5-^{3}H(N)]galactose, NEN) or glucosamine (D-

[1,6-^{3}H(N)glucosamine, NEN) is then be added to each flask and
the cultures allowed to incubate for an additional 30-40
hours. Cells are harvested by scraping and EBs purified by
gradient centrifugation. HMW protein is isolated from 1.0%
5 OGP surface extracts by affinity chromatography, eluted with
NaCl and analyzed by SDS-PAGE using ^{14}C-labelled molecular
weight markers (BRL) then subjected to autoradiography.
Dried gels are exposed for 1-4 weeks to Kodak X-AR film at -
70°C.

10   To determine post synthesis lipid modification,
C. trachomatis serovar L$_2$ is cultivated on monolayers of McCoy
cells according to standard procedures. Approximately 24
hours postinfection, conventional culture media (DMEM + 10%
FCS) is removed and replaced with a serum-free medium
15 containing cycloheximide (1 μg/ml) and [U-^{14}C]palmitic acid
(0.5 mCi/T225 flask, NEN) and incubated for a further 16-24
hours to allow protein lipidation to occur. Surface EB
extracts are prepared, heparin-binding proteins are isolated
and analyzed by autoradiography as described above.

20   The functionality of glycosylated or lipidated
moieties is assessed by treating whole EBs or OGP surface
extracts with appropriate glycosidases. Following
carbohydrate removal, extracts are subjected to affinity
chromatography and SDS-PAGE to determine whether the HMW
25 protein retains the ability to bind to heparin sulfate.

8.4. EXAMPLE 4: Cloning of the N-terminal Segment of the
HMW Protein Gene
          Degenerate oligonucleotides were designed based on
30 the N-terminal amino acid sequence of the HMW protein and
were synthesized. These oligonucleotides were then used to
generate gene-specific PCR products that were employed as
hybridization probes to screen a C. trachomatis L$_2$ λZAPII DNA
library to isolate the gene for the HMW protein.
35   Briefly, appropriate low degeneracy peptide
segments were identified from the N-terminal and internal
amino acid sequence data by computer analysis (MacVector,

IBI) and used to guide the design of low degeneracy sequence-specific oligonucleotide PCR primer sets.

Using the N-terminal primary sequence as a guide, four degenerate oligonucleotide probes complementary to the
5 first six residues of the HMW peptide E-I-M-V-P-Q (residues 1-6 of SEQ ID No.: 3), and comprising all possible nucleotide combinations (total degeneracy = 192 individual sequences), have been designed and employed as forward amplification primers.
10

        SEQ ID No.4   5'-GAA-ATH-ATG-GTN-CCN-CAA-3'.
        SEQ ID No.5   5'-GAA-ATH-ATG-GTN-CCN-CAG-3'
        SEQ ID No.6   5'-GAG-ATH-ATG-GTN-CCN-CAA-3'
        SEQ ID No.7   5'-GAG-ATH-ATG-GTN-CCN-CAG-3'
15

Two additional oligonucleotide probes representing the reverse complement DNA sequence of the internal five residue peptide Y-D-G-E-T (residues 9-13 of SEQ ID No.: 3), and comprising all possible nucleotide combinations (total
20 degeneracy = 128 individual sequences), have been designed and employed as reverse amplification primers.

        SEQ ID No.8   5'-NGT-YTC-NCC-RTC-ATA-3'
        SEQ ID No.9   5'-NGT-YTC-NCC-RTC-GTA-3'
25

        Oligonucleotides were synthesized on an ABI Model 380B DNA synthesizer using a 0.2 μmol scale column (trityl-on, auto-cleavage) and standard phosphoramidite chemistry. Crude oligonucleotides were manually purified over C-18
30 syringe columns (OP Columns, ABI). Purity and yield were ascertained spectrophotometrically (230/260/280 ratios).
        Standard PCR amplification reactions (2 mM Mg^{2+}, 200 umol dNTPs, 0.75 units AmpliTaq, 50 μl final volume) were programmed using about 0.2 μg C. trachomatis L_2 DNA (about
35 $3X10^7$ copies of the HMW protein gene if single copy) and about 100 pmol of each forward (N-terminal oligo) and reverse (internal oligo) primer. Higher than normal concentrations

of primers (~20 pmol/50 μl) were used for amplification, at
least initially, in order to compensate for primer
degeneracy. Amplification of target sequences was achieved
using a standard 30-cycle, three-step thermal profile, i.e.
5 95°C, 30 sec; 60°C, 45 sec, 72°C, 1 min. Amplification was
carried out in sealed 50 μl glass capillary tubes using a
Idaho Technologies thermal cycler. To verify that the PCR
products generated during these amplification reactions were
specific for the HMW protein coding sequence and were not
10 "primer-dimer" or other DNA amplification artifacts,
amplimers were purified using silica-gel spin columns
(QIAGEN), cloned into the PCR cloning vector pZERO
(StrataGene), and subjected to direct DNA sequence analysis.
        The DNA sequence for the cloned PCR products were
15 determined using conventional dideoxy-terminator sequencing
chemistry and a modified T7 DNA polymerase (Sequenase, USB).
Briefly, each double stranded plasmid template was denatured
by a brief treatment with NaOH. Following neutralization,
each denatured template was used to program 4 separate
20 sequencing reactions. Each reaction contained the M13
universal forward sequencing primer (21-mer) but a different
ddNTP/dNTP termination mix (i.e. A,G,C, or T). Termination
products were labelled by including [α-^{35}S]dATP in the
reaction (~50uCi/reaction, >3000Ci/mmol, Amersham).
25 Individual extension products were denatured (formamide,
~95°C) and subjected to high resolution denaturing
polyacrylamide gel electrophoresis (6% acrylamide, 8M urea,
TAE buffer, ~500V, ~90min). Sequencing gels were then
transferred to filter paper (Whatmann 3MM), dried under
30 vacuum, and then autoradiographed at -70°C for 24-72 hours.
Base ladders were read manually from each gel and a consensus
sequence determined.
        HMW protein-specific amplimers suitable for library
screening and/or Southern blotting were produced by PCR and
35 uniformly radiolabelled during the amplification process by
adding [α-^{32}P]dNTPs (about 50 μCi each dNTP, Amersham, >5000
Ci/mmol) to the reaction mixture. Labelling reactions were

performed as above except reactions were performed in 0.5 ml microcentrifuge tubes using a Bellco Thermal Cycler. Unincorporated label and amplification primers were removed from the reaction mixture using centrifugal size-exclusion
5   chromatography columns (BioSpin 6 columns, BioRad).

A highly redundant *C.trachomatis* serovar L_2 DNA library (>50,000 primary clones) has been constructed by cloning size-fractionated fragments >10 Kbp produced from a partial EcoRI digest of genomic DNA into the lambda cloning
10 vector λZAPII (Stratagene). Radiolabelled HMW protein-specific PCR products were used to screen this library for recombinant clones that carry all or part of the HMW protein coding sequence. Standard recombinant DNA procedures and methodologies were employed for these experiments. All phage
15 that hybridized with these probes were purified to homogeneity by sequential rounds of plating and hybridization screening. Once reactive phage were purified, insert-containing phagmids (pBluescript SK- derivatives) were excision-rescued from the parental phage by coinfecting host
20 cells with an appropriate helper phage, *e.g.* R408 or VCSM13 (Stratagene). Individual phagmids were further purified by streak-plating on LB agar containing ampicillin (100 μg/ml) and selecting for individual colonies.

To confirm purified phagemid derivatives carried
25 the HMW protein sequences, plasmid DNA was prepared and used to program amplification reactions containing the HMW protein-specific PCR primer sets. The presence of HMW protein-specific inserts was confirmed by the production of the appropriate sized PCR product.
30   Plasmid pAH306 is one HMW protein-containing derivative that was isolated by these methodologies.

Physical Mapping of pAH306

The inserts from pAH306 were physically mapped and
35 the location of HMW protein gene determined using appropriate six-base restriction endonucleases (*e.g.* EcoRI, HindIII, BamHI, PstI, SmaI, KpnI, etc.) and HMW protein coding

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sequences localized by Southern hybridization employing
radiolabelled N-terminal-specific PCR products as probes.
The orientation and extent of HMW protein-specific sequences
were determined by PCR analysis using primer sets consisting
5 of HMW protein-specific forward primers and reverse primers
complementary to either the T3 or T7 promoter sequences
located in the cloning vector.

Plasmid pAH306 was determined to contain a single
~6.6 Kbp EcoRI fragment of chlamydial origin. Directional
10 PCR analysis of pAH306 demonstrated this derivative encodes
roughly 1.5Kbp of the N-terminal region of the HMW protein
gene.

The DNA sequence for the HMW protein gene encoded
on pAH306 was obtained for both strands via conventional
15 "sequence-walking" coupled with asymmetric PCR cycle
sequencing methodologies (ABI Prism Dye-Terminator Cycle
Sequencing, Perkin-Elmer). Sequencing reactions were
programmed with undigested plasmid DNA (~0.5 μg/rxn) as a
template and appropriate HMW protein-specific sequencing
20 primers (~3.5 pmol/rxn).

In addition to the template and sequencing primer,
each sequencing reaction (~20 μl) contained the four
different dNTPs (i.e. A,G,C, and T) and the four
corresponding ddNTPs (i.e. ddA, ddG, ddC, and ddT) terminator
25 nucleotides; with each terminator being conjugated to one of
four different fluorescent dyes. Single strand sequencing
elongation products were terminated at random positions along
the template by the incorporation of the dye-labelled ddNTP
terminators. Fluorescent dye-labelled termination products
30 were purified using microcentrifuge size-exclusion
chromatography columns (Princeton Genetics), dried under
vacuum, suspended in a Template Resuspension Buffer (Perkin-
Elmer), denatured at 95°C for ~5min, and resolved by high
resolution capillary electrophoresis on an ABI 310 Automated
35 DNA Sequenator (Perkin-Elmer).

DNA sequence data produced from individual
reactions were collected and the relative fluorescent peak

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intensities analyzed automatically on a PowerMAC computer
using ABI Sequence Analysis Software (Perkin-Elmer).
Individually autoanalyzed DNA sequences were edited manually
for accuracy before being merged into a consensus sequence

5 "string" using AutoAssembler software (Perkin-Elmer). Both
strands of the HMW protein gene segment encoded by pAH306
were sequenced and these data compiled to create a composite
sequence for the HMW protein gene segment. The sequence
encoding the segment of HMW protein is listed as SEQ ID No.:

10 10 and is represented by nucleotides 382 to 1979 in Figure 2.
A map of pAH306 is shown in Figure 5.

Database analysis (e.g. primary amino acid
homologies, hydropathy profiles, N-/O-glycosylation sites,
functional/conformational domain analyses) of the DNA and

15 predicted amino acid sequences for the HMW protein was
performed using GeneRunner and Intelligentics software,
indicating the HMW protein is novel.

8.5. Example 5:   CLONING OF THE C-TERMINAL SEGMENT OF THE

20 HMW PROTEIN GENE

Chromosome walking was employed to isolate the C-
terminal portion of the HMW protein gene. A ~0.6 Kbp BamHI-
EcoRI fragment distal to the N-terminal sequence of the
mature HMW protein and proximal to the T3 promoter sequence

25 of the vector was chosen as the probe for the initial
chromosome walk. Briefly, pAH306 was digested to completion
with BamHI and EcoRI and the digestion products size
fractionated by agarose gel electrophoresis (0.8% agarose in
TAE buffer). The desired ~0.6 Kbp BamHI/EcoRI (B/E) band was

30 excised from the gel and purified using commercially
available silica gel microcentrifuge chromatography columns
and reagents (QIAGEN).

The purified 0.6 Kbp B/E fragment was radiolabelled
with [α-dATP] (>3000Ci/mmol, Amersham) via random-priming

35 labelling methodologies employing commercially available
reagents (Boehringer Mannheim) and used to probe Southern

blots of *C. trachomatis* L_2 genomic DNA that had been digested to completion with HindIII.

The 0.6 Kbp B/E probe from pAH306 hybridized to a ~1.4Kbp HindIII genomic fragment. Based on the experimentally
5 derived restriction map of the HMW protein gene segment encoded on pAH306, this fragment encodes ~0.2Kbp of the C-terminal HMW protein sequence.

The radiolabelled 0.6 Kbp B/E fragment was used subsequently to probe a moderately redundant (~5,000 primary
10 clones) *C. trachomatis* L2 library to identify clones that contain the ~1.4Kbp HindIII fragment. Briefly, *C. trachomatis* L_2 genomic DNA was digested to completion using a ~10-fold excess of the restriction endonuclease HindIII (~10 units per 1 μg of genomic DNA, 37°C, 18-24 hours). Digestion
15 products were size fractionated by agarose gel electrophoresis (0.8% agarose, TAE) and DNA fragments ranging in size from ~1.0 Kbp to 2.0 Kbp were excised from the gel. Excised agarose strips contain the desired DNA fragment sizes were dissolved in a solubilization/binding solution
20 (QX1,QIAGEN) and purified using commercially available silica-gel spin columns (QIAGEN). Purified 1.0-2.0 Kbp genomic HindIII fragments were then cloned into the pBlueScript SK- plasmid which had been previously digested to completion with HindIII and treated with calf intestinal
25 phosphatase to prevent vector religation.

Vector/insert ligations were performed in a ~50 μl final reaction volume (50 mM Tris-HCl, pH 7.00; 10 mM NaCl; 1 mM ATP; 0.5 mM DTT) at 25°C for ~16-24 hours using T4 DNA ligase (~10 units/reaction) and a vector:insert molar ratio
30 of approximately 1:10. Following ligation, aliquots (~50 ng ligated DNA) were used to electroporate a competent *E.coli* host, *e.g. E.coli* TOP10. Electroporated cells were then plated onto LB agar containing ~100μg/ml ampicillin to select for plasmid-harboring clones. Approximately 1,000 plasmid-
35 harboring Ap^R transformants were transferred directly from LB Ap^{100} agar plates onto nylon membranes (HyBond N+, Amersham) by capillary action.

Following transfer, plates were re-incubated at
37°C to regenerate viable colonies for further manipulation.
Colonies transferred to membranes were lysed and DNA
liberated by treating the colony blots with a denaturing
5   SDS/NaOH solution. A Tris buffered NaCl solution was used to
neutralize and stabilize lysis material. Released DNA was
immobilized onto the membranes by UV irradiation. Standard
recombinant DNA procedures and methodologies were employed to
probe the colony blots with the radiolabelled 0.6 Kbp B/E
10 fragment and identify recombinant derivatives which carry the
desired ~1.4Kbp HindIII fragment.

Plasmid pAH310 was one derivative isolated by these
procedures and the coding segment of the HMW protein is
represented by nucleotides 994-2401 in Figure 2.
15   Restriction analysis using HindIII and EcoRI,
individually and in combination, together with DNA sequence
analysis of purified plasmid DNA confirmed pAH310 encodes the
expected ~1.4 Kbp HindIII fragment. These analyses also
demonstrated that the ~1.4 Kbp insert consists of the same
20 ~1.2 Kbp HindIII-EcoRI fragment that is present in pAH306 and
a unique ~0.2 Kbp EcoRI-HindIII fragment that encodes C-
terminal HMW protein-specific DNA.

The ~0.2 Kbp EcoRI-HindIII (E/H) fragment was
chosen as the probe for the second chromosome walk. Briefly,
25 pAH310 was digested to completion with EcoRI and HindIII and
the digestion products size fractionated by agarose gel
electrophoresis (0.8% agarose in TAE buffer). The desired
~0.2 Kbp (E/H) band was excised from the gel, purified,
radiolabelled with [α-P^{32}]dATP, and used as a probe to
30 identify clones in the original *C.trachomatis* L$_2$ λZAPII
genomic library that encode the C-terminal segment of the HMW
protein gene.

Plasmid pAH316 is one derivative isolated by these
procedures. Restriction analysis of pAH316 demonstrated that
35 this derivative contains a *C. trachomatis* L$_2$ insert of ~4.5
Kbp which consists of two EcoRI fragments of ~2.5 Kbp and
~2.0 Kbp in size. Southern hybridization analysis using the

- 50 -

~0.2 Kbp E/H fragment as a probe localized this sequence to
the ~2.5 Kbp EcoRI fragment of pAH316. Directional PCR
analyses employing purified pAH316 plasmid DNA as a template
and amplification primer sets specific for ~0.2 Kbp E/H
5   fragment and T3 and T7 vector sequences demonstrated pAH316
encodes the C-terminal segment of the HMW protein gene. The
coding segment of the HMW protein is represented by
nucleotides 1974 to 3420 in Figure 2, and is listed as SEQ ID
No.:11.
10

8.6. Example 6: PRODUCTION OF TRUNCATED HMW RECOMBINANT
PROTEIN
        The N-terminal half of the HMW protein was PCR
cloned as a ~1.5Kbp fragment into the commercially available
15   *E.coli* expression plasmid pTrcHisB (Invitrogen). The forward
primer used in these reactions was designated 140FXHO (57-
mer), listed as SEQ ID No. 18, and contains sequences
complementary to the first 10 N-terminal residues of the
mature HMW protein. In addition to the HMW proteincoding
20   sequences, this forward primer also carries a unique XhoI
restriction site located optimally located upstream of the
first residue of the mature HMW protein (Glu/E) for proper
fusion to the (His)$_6$ affinity purification domain encoded on
the vector plasmid, and 5' terminal 6 base G/C clamp for
25   effective amplification and a 12 base internal spacer for
effective endonuclease recognition and digestion.

SEQ ID No.18   5' - AAG-GGC-CCA-ATT-ACG-CAG-AGC-TCG-AGA-GAA-
ATT-ATG-GTT-CCT-CAA-GGA-ATT-TAC-GAT - 3'
30
SEQ ID No.19   5' - CGC-TCT-AGA-ACT-AGT-GGA-TC - 3'

The commercially available reverse sequencing primer SK
(20mer, StrataGene), SEQ ID No. 19, which is complementary to
35   phagemid sequences downstream of the EcoRI site in pAH306,
was used as the reverse amplification primer in these
reactions. To obtain acceptable yields of the HMW protein ORF

product (~1.5 Kbp), PCR amplification was performed using a
mixture of thermostable DNA polymerases consisting of *T.
thermophilus* DNA polymerase (Advantage Polymerase), as the
primary amplification polymerase and a minor amount of a
5 second high fidelity thermostable DNA polymerase to provide
additional 5' - 3' proofreading activity (CloneTech). An
anti-Tth DNA polymerase antibody was added to the reaction
mixture to provide automatic "hot-start" conditions which
foster the production of large >2Kbp amplimers. pAH306
10 plasmid DNA purified using a commercially available
alkaline/SDS system (QIAGEN) and silica gel spin columns
(QIAGEN) was used to program these amplification reactions
(~0.2 ng/reaction).

The ~1.5 Kbp amplimer was purified from
15 unincorporated primers using silica gel spin columns and
digested to completion using an excess of XhoI and EcoRI (~10
units per 1 μg DNA). The purified and digested N-terminal
truncated HMW protein ORF was then be cloned into the
commercially available expression plasmid pTrcHisB that had
20 been previously digested with both XhoI and EcoRI (5:1,
insert:vector ratio). Aliquots from the ligation reaction
were then be used to electrotransform a suitable *E.coli* host
(*e.g.* TOP10).

Mini-prep DNA from ampicillin-resistant
25 transformants picked at random were prepared, digested to
completion with XhoI, EcoRI, or a combination of both and
examined for the presence and orientation of the ~1.5 Kbp
truncated HMW protein ORF insert by agarose gel
electrophoresis. Mini-prep DNA from clones determined to
30 carry the ~1.5 Kbp XhoI/EcoRI insert was prepared and used to
program asymmetric PCR DNA sequencing reactions to confirm
the fidelity of the junction formed between the HMW protein
fragment and the (His)$_6$ affinity purification domain of the
expression vector. Plasmid pJJ36-J was one recombinant
35 derivative isolated by these procedures and is represented by
nucleotides 446 to 1977 in figure 2. The deduced amino acid
sequence of the truncated fragment of HMW protein is

represented by amino acids 29 to 532 in Figure 3 and is
listed as SEQ ID No. 17.

8.7. EXAMPLE 7: DETERMINATION OF PRESENCE IN OTHER SPECIES

5   Polymerase chain reaction analyses were undertaken
to establish the presence of the HMW gene in several
clinically recognized *C. trachomatis* strains and as well as
other chlamydial species, *e.g.*, *C. pneumoniae*. *Chlamydia
trachomatis* strains as frozen stocks from the ATCC
10 (Rockville, MD) were used to infect subconfluent monolayers
(about 80%) of McCoy cells according to standard procedures.
Infected monolayers were either centrifuged in a Sorvall
RT6000B centrifuge (~1,300 rpm, 25°C, 30 min) and/or treated
with dextran sulfate (~50 μg/ml) at the time of infection to
15 enhance initial attachment of the low infectivity biovars
(non-LGV) to host cells and thus increase the final EB yield.
Roughly 48 hours later, infected monolayers were collected by
scraping and host cells disrupted by sonication to release
elementary bodies (EBs). Total DNA was extracted from
20 purified EBs (~10^7-10^8) of each strain using the proteinase K/
Nonidet P40 method described by Denamur, et al., J. Gen.
Microbiol. 137:2525-2530 (1991), incorporated herein by
reference, and further purified by phenol/chloroform
extraction and salt precipitation. Purified *Chlamydia
pneumoniae* (AR-139) genomic DNA was purchased from Advanced
Biotechnologies Inc.

          To determine the presence of the HMW protein gene
in these strains, amplification reactions were programmed
using total *Chlamydia* DNA as template and the HMW protein
30 segment-specific oligonucleotide primer (21mers) sets listed
below.

SEQ ID No.20   5' - ATG-GTT-CCT-CAA-GGA-ATT-TAC-G - 3'
SEQ ID No.21   5' - GGT-CCC-CCA-TCA-GCG-GGA-G - 3'

          Briefly, standard PCR amplification reactions (2 mM
35 Mg^{2+}, 100 μmol dNTPs, 0.75 units AmpliTaq polymerase, 50 μl
final volume) were programmed using approximately 15 μl of
the crude *C.trachomatis* DNA extracts (~10 μl of the

commercially available *C. pneumoniae* DNA) and ~20 pmol of
each forward and reverse HMW protein-specific amplification
primers of SEQ ID No. 20 and 21. Amplification of small
target sequences (≤ 2 Kbp) was achieved using a 32-cycle,
5   three-step thermal profile, *i.e.* 95°C, 30 sec; 60°C, 30 sec,
72°C, 1 min. Amplification of longer target sequences for
ORF-cloning and sequencing was carried out using the crude
DNA extracts in an identical fashion except that a MAb-
inactivated Tth/Vent DNA polymerase enzyme combination was
10 employed (Advantage PCR, Clontech) and a 72°C extension time
was used that matched the size of the desired PCR product
plus 2 min (*i.e.* desired PCR product = 6Kbp, extension time =
8 min).

        Both conventional and long-distance PCRs were
15 carried out using 0.2 ml thin-walled polypropylene
microcentrifuge tubes in an ABI 2400 Thermal Cycler (Perkin-
Elmer). Following thermal cycling, aliquots (~20 μl) of the
reactions were analyzed and PCR products identified by
standard agarose gel electrophoresis (0.8% agarose in TAE
20 buffer) and ethidium bromide staining. The results showed
that the HMW protein is highly conserved in clinically
relevant serovars; the HMW gene was present in all *C hlamydia
samples* strains tested, including serovars B, Ba, D, E, F, G,
H, I, J, K, L$_1$, L$_2$ and MoPn and in *C. pneumoniae*.
25

8.8.   EXAMPLE 8:   DETERMINATION OF SEQUENCE VARIATION
        To establish the degree of DNA and amino acid
sequence variation among different *Chlamydia* strains, the
gene for the HMW protein was PCR-cloned from both a *C.*
30 *trachomatis* B serovar (representing the trachoma group of
organisms) and from a *C. trachomatis* F serovar (representing
the low infectivity STD biovars) and compared to the HMW
protein consensus *C. trachomatis* L$_2$ sequence.
        Briefly, LD-PCR was used to generate ~6Kbp HMW
35 protein-specific DNA fragments from *C. trachomatis* B and F
genomic DNA that contain the complete coding sequence for the
mature HMW protein. Amplification conditions for these LD-

PCR exercises were as described in Example 6. The reverse
amplification primer employed in these reactions (p316Kpn-RC,
56mer), listed as SEQ ID No. 13, is complementary to a
sequence located ~3 Kbp downstream of the predicted HMW

5 protein termination codon. As an aide to cloning the desired
~6 Kbp amplimer, a single KpnI restriction endonuclease site
5' to the chlamydial sequence was engineered into the
p316Kpn-RC primer. The forward amplification primer used for
these reactions (p306Kpn-F, 56mer), listed as SEQ ID No. 12,

10 contains the sequence complementary to the first 10 amino
acid residues (30 nucleotides) specifying the mature HMW
protein as well as a 5' sequence specifying a KpnI site.
p306Kpn-F was designed such that the sequence encoding the N-
terminus of the mature HMW protein could be linked in-frame

15 to a hexa-His affinity domain encoded downstream of the
highly efficient trc promoter on the *E.coli* expression vector
pTrcHisB (ClonTech) when the ~6 Kbp amplimer was inserted
into the KpnI site of this vector.

20 SEQ ID No.12   5'-AAG-GGC-CCA-ATT-ACG-CAG-AGG-GTA-CCG-AAA-
TTA-TGG-TTC-CTC-AAG-GAA-TTT-ACG-AT-3'

SEQ ID No.13   5' -AAG-GGC-CCA-ATT-ACG-CAG-AGG-GTA-CCC-TAA-
GAA-GAA-GGC-ATG-CCG-TGC-TAG-CGG-AG- 3'

25

The ~6 Kbp HMW protein products were purified using silica-
gel spin columns (QIAGEN) and the fragments subjected to two
8-10 hour cycles of KpnI digestion using a 10-fold excess of
KpnI (~10 units per 1 μg of purified fragment, 37°C).

30 Following the second digestion, residual restriction enzyme
activity was removed using QIAGEN spin columns and the ~6 Kbp
KpnI HMW protein fragments cloned into the pTrcHisB plasmid
which had been previously digested to completion with KpnI
and treated with calf intestinal phosphatase to prevent

35 vector religation.

        Vector/insert ligations were performed in a ~50 μl
final reaction volume (50 mM Tris-HCl, pH 7.00; 10 mM NaCl; 1

mM ATP; 0.5 mM DTT) at 25°C for ~2 hours using T4 DNA ligase
(~10 units/reaction) and a vector:insert molar ratio of
approximately 1:5. Following ligation, aliquots (~50 ng
ligated DNA) was used to electroporate a competent *E.coli*

5 host, *e.g. E.coli* TOP10. Plasmid-harboring transformants
were selected by plating electrotransformed cells onto LB
agar containing 100 µg/ml ampicillin. Ampicillin-resistant
(Ap^R) transformants appearing after a ~18-24 hour incubation
period at 37°C were picked at random and restreaked onto the

10 same selective media for purification.

A single, purified Ap^R colony from each initial
transformant was used to inoculate ~5ml of LB broth and grown
overnight at 37°C in a incubator shaker with mild aeration
(~200 rpm). Cells from broth cultures were harvested by

15 centrifugation and used to prepare small quantities of
plasmid DNA. Commercially available reagents (QIAGEN Plasmid
Mini Kits) were employed for these plasmid extractions.
Plasmid derivatives carrying inserts were presumptively
identified by electrophoresing the non-digested plasmid DNA

20 in agarose gels (0.8% agarose in TAE buffer)and identifying
derivatives greater in size than vector plasmid. Insert-
containing derivatives were confirmed and the orientation of
the HMW protein inserts relative to vector sequences were
determined using appropriate restriction endonucleases (KpnI,

25 EcoRI, HindIII, BamHI, etc.), either separately or together
in various combinations.

The DNA sequence of the *C. trachomatis* B and F HMW
protein genes were obtained for both strands using "sequence
walking" the asymmetric dye-terminator PCR cycle sequencing

30 methodology (ABI Prism Dye-Terminator Cycle Sequencing,
Perkin-Elmer) described in Example 4. Reactions were
programmed with plasmid mini-prep DNA and individual HMW
protein sequence-specific primers that were employed in the
sequencing of the HMW protein gene from the L$_2$ type strain.

35 DNA sequence data were collected using the ABI 310
Sequenator and analyzed automatically on a PowerMAC computer
and appropriate computer software as described in Example 4.

Individually autoanalyzed DNA sequences were edited manually
for accuracy before being merged into a consensus sequence
"string" using AutoAssembler software (Perkin-Elmer). Both
strands of the HMW protein gene from the *C. trachomatis* B and

5   F serovars were sequenced and these data compiled to create
composite consensus sequences for both the *C. trachomatis* B
and F HMW protein genes. These sequences are listed as SEQ
ID Nos.: 14 and 15. Sequence comparisons of the L_2, F and B
strains are presented in Figure 6.

10

8.9. **EXAMPLE 9:** PRODUCTION OF RECOMBINANT PROTEIN

To produce sufficient quantities of recombinant HMW
protein for both immunogenicity and animal protection
studies, the HMW gene has been PCR cloned into suitable

15 *E.coli* and baculovirus expression systems. Large quantities
of rHMW protein are produced in an *E.coli* - based system as a
chimeric fusion protein containing an N-terminal $(His)_6$
affinity purification domain. The complete HMW protein open
reading frame (ORF) was PCR-cloned from the *C. trachomatis* L_2

20 genome as a single KpnI fragment and fused in the proper
orientation and in the correct reading frame to the $(His)_6$
affinity purification domain encoded on the high expression
plasmid vector pTrcHisB (CloneTech) as described in Example
5.

25   The $(His)_6$ affinity purification domain is part of a
high expression locus consisting of the highly efficient *tac*
promoter (IPTG-inducible) and consensus Shine and Delgarno
ribosome binding site (RBS) located immediately upstream of
the $(His)_6$ affinity purification domain. The HMW protein

30 genes from *C. trachomatis* LGV L_2, *C. trachomatis* B, and
C.trachomatis F were PCR cloned as ~3.0 Kbp fragments. The
forward primer (56-mer) used in these reactions was
designated p306Kpn-F and contains sequences complementary to
the first 10 N-terminal amino acid residues of the mature HMW

35 protein, listed as SEQ ID No 12. In addition to the HMW
protein coding sequences, this forward primer also carries a
unique KpnI restriction site located optimally located

upstream of the first residue of the mature HMW protein(Glu) for proper fusion to the (His)$_6$ affinity purification domain encoded on the vector plasmid, and 5' terminal 6 base G/C clamp for effective amplification and a 12 base internal

5   spacer for effective endonuclease recognition/digestion. The reverse PCR primer, designated p316Kpn-3RC, contains a reverse complement sequence to a *C. trachomatis* sequence located ~0.2Kbp downstream of the HMW protein termination codon, listed as SEQ ID No. 14. As with p306Kpn-F, the

10 reverse primer also contains a KpnI restriction site 5' to the *C. trachomatis* sequences, a 6 base G/C clamp, and a 12 base internal spacer.

        To obtain acceptable yields of the HMW protein ORF product (about 3,500bp), PCR amplification was performed

15 using a mixture of thermostable DNA polymerases consisting of *T. thermophilus* DNA polymerase as the primary amplification polymerase and a minor amount of a second high fidelity thermostable DNA polymerase to provide additional 5'-3' proofreading activity (Advantage Polymerase, CloneTech). An

20 anti-Tth DNA polymerase antibody was added to the reaction mixture to provide automatic 'hot-start' conditions which foster the production of large (>2 Kbp) amplimers.

        Genomic DNA from the various *C.trachomatis* strains was isolated from EBs as described in the example above and

25 used to program these reactions. Following amplification, the desired reaction products were purified from excess primers using commercially available silica-gel spin columns and reagents (QIAGEN) and digested to completion with an excess of KpnI (~10 units per 1μg DNA). The purified and

30 digested KpnI HMW protein ORF was then be cloned into the KpnI predigested pTrcHisB expression plasmid (5:1, insert:vector ratio). Aliquots from the ligation reaction were then used to electrotransform a suitable *E.coli* host (e.g. TOP10).

35   Mini-prep DNA from ampicillin-resistant transformants picked at random were prepared, digested to completion with KpnI, HindIII, or a combination of both and

examined for the presence and orientation of the ~3.2 Kbp HMW
protein ORF insert by agarose gel electrophoresis and
ethidium bromide staining. Mini-prep DNA was used to program
asymmetric PCR DNA sequencing reactions as described in
5 example(s) above to confirm the fidelity of the junction
formed between the HMW protein fragment and the (His)$_6$
affinity purification domain of the vector. Plasmid pAH342
was one derivative isolated by these procedures, which
contains the HMW protein gene ORF from *C. trachomatis* L$_2$ and
10 is represented by nucleotides 446 to 3421 in Figure 2.

Recombinants were grown in 2X-YT broth containing
100 μg/ml Ap to mid-log phase (~0.5 O.D.$_{600}$) and induced with
IPTG (1mM final) for an additional 4-5 hours to activate
transcription from the vectors *trc* promoter. Cells were
15 harvested by centrifugation and crude cell lysates prepared
by lysis using a French pressure cell.

Alternatively, expression of rHMW protein may be
obtained by using a baculovirus expression system. Here, the
HMW protein ORF from *C. trachomatis* L$_2$ and *C. trachomatis* F were
20 PCR-cloned as ~3 Kbp PCR products into a baculovirus transfer
vector (*e.g.* pFastBacHTb) that had been previously digested
to completion with KpnI and treated with CIP to minimize
vector religation in essentially the same manner as described
for pTrcHisB. The HMW protein expression cartridge generated
25 in this cloning exercise (*i.e.* the baculovirus polyhedron
promoter, N-terminal (His)$_6$ affinity purification domain, HMW
protein gene ORF) was then transferred to the baculovirus
genome by site-specific transposition using a commercially
available bacmid system (Bac-to-Bac, Gibco)
30 Briefly, the HMW protein baculovirus expression
plasmid was used to transform competent *E. coli* DH10bac
(Gibco) cells containing a bacmid (a hybrid baculovirus-
plasmid replicon) to gentamicin resistance using standard
transformation and selection methodologies. Transformants
35 where the HMW protein expression cartridge had successfully
transposed from the expression plasmid to the appropriate
receptor site within the lacZ gene located on the bacmid

replicon were identified using a standard IPTG/X-gal blue-white selection.

White, Gm[R] transformants were picked at random and restreaked for purification. Bacmid DNA was prepared from
5 broth cultures by the method of Ish-Horowitz, N. A. R. 9:2989-2993 (1981) incorporated herein by reference, and is used to transfect *Spodoptera frugiperda* 9 cells. Following plaque purification, recombinant HMW protein baculovirus is used to infect large scale *Spodoptera* suspension cultures. A
10 yeast expression system is used to generate a glycosylated form of HMW protein.

8.10.   EXAMPLE 10:   PURIFICATION OF RECOMBINANT PROTEIN

Recombinant HMW protein was purified to homogeneity
15 using standard preparative immobilized metal affinity chromatography (IMAC) procedures. Briefly, an *E. coli* strain harboring an expression plasmid containing HMW protein gene was grown in Luria broth in a 5l fermenter (New Brunswick) at 37°C with moderate aeration until mid-log phase (~ 0.5 O.D.$_{600}$)
20 and induced with IPTG (1mM final) for 4-5 hours. Cell paste was collected, washed in PBS and stored at -20°C. Aliquots of frozen cell paste (~ 9-10 grams wet weight) were suspended in ~ 120 ml of D-PBS by mechanical agitation and lysed by passage through a French pressure cell (2X, 14,000psi, 4°C).
25 Soluble protein was then removed from rHMW protein inclusion bodies by high speed centrifugation ($\sim 10,000$Xg, 4°C, 30min).

The insoluble pellet containing rHMW protein was suspended in ~ 20ml of ice cold D-PBS by homogenization and centrifuged as above. Washed rHMW protein inclusion bodies
30 were then denatured by suspension in a sodium phosphate buffer (0.1 M, pH 7.0) containing 6M guanidine hydrochloride and loaded onto a Ni^{2+}-affinity column (1.5 cm X 25 cm, bed volume ~ 15 ml) prepared from Fast-Flow Chelating Sepharose (Pharmacia) and charged with Ni^{2+}or Zn^{2+} ions by standard
35 procedures. Unbound material was removed by washing the column with ~ 5-10 column volumes of a sodium phosphate buffer (0.1 M, pH 7.0) containing 8M urea.

Recombinant HMW protein bound to the affinity resin
by virtue of the N-terminal (His)$_6$ affinity purification
domain was eluted using a pH 4.0 sodium phosphate/8M urea
buffer (~20 ml). Eluted material was neutralized by the
5 addition of 1.0 M Tris-HCl (~2.5 ml, pH 7.5) and dialyzed
against TE buffer containing SDS (0.5%) to remove the urea.
Dialyzed material was concentrated using a Centricon-30
centrifugal concentrator (Amicon, 30,000 MWCO) and mixed with
a 1/5 volume of 5X SDS gel sample buffer containing 1 mM 2-
10 mercaptoethanol (Lammeli) and boiled at 100°C for 5 minutes.
Samples were loaded onto Tris/glycine preparative
acrylamide gels (4% stacking gel, 12% resolving gel, 30:0.8
acrylamide:bis solution, 3mm thickness). A prestained
molecular weight standard (SeeBlue, Novex) was run in
15 parallel with the rHMW protein samples to identify size
fractions on the gel. The area of the gel containing
proteins having molecular masses of ~50-70 Kdal was excised
and the proteins electroeluted using an Elu-Trap device and
membranes (S&S) as specified by the manufacturer.
20 Electroeluted protein was dialyized to remove SDS. The
protein concentration of the sample was determined using a
Micro-BCA system (Pierce) and BSA as a concentration
standard. The purity of rHMW protein was determined using
conventional SDS-PAGE and commercially available silver
25 staining reagents (Silver Stain Plus, Novex) as shown in
Figure 4.
The apparent molecular weight of the isolated
mature rHMW is about 105-115 kDa as determined by SDS-PAGE.

30 **8.11.   EXAMPLE 11:   PREPARATION OF ANTIBODIES TO HMW PROTEIN**
Polyvalent antisera directed against the HMW
protein were generated by vaccinating rabbits with the
purified HMW protein or fragments thereof. Each animal was
given a total of three immunizations of about 250 μg HMW
35 protein or fragment thereof per injection (beginning with
complete Freund's adjuvant and followed with incomplete
Freund's adjuvant) at approximately 21 day intervals. At

each immunization, approximately half of the material was
administered intramuscullarly (i.m.) and half was injected
intranodally. Fourteen days after the third vaccination a
fourth booster of about 100 μg HMW protein was given i.m. and
5 the animals exsanguinated 7-10 days later. Anti-HMW protein
titers were measured by ELISA using purified HMW protein (1.0
μg/well) or *C. trachomatis* L$_2$ EBs (whole and crude protein
extracts) as capture ligands. Immunogen specific IgG ELISA
titres of 1/320,000 were observed using purified rHMW
10 truncated protein and 1/2500 using either EBs or RBs.

Serial dilutions of antisera were made in PBS and
tested by ELISA in duplicate. Goat HRP-conjugated anti-
rabbit antibody diluted 1/1000 was used as the second
reporter antibody in these assays. Titers are expressed as
15 the greatest dilution showing a positive ELISA reaction, *i.e.*
an O.D.$_{450}$ value >2SD above the mean negative control value
(prebleed rabbit sera). Hyperimmune antisera was then used
to probe Western blots of crude EB or RB extracts as well as
1.0% OGP EB extract preparations to determine whether other
20 *C. trachomatis* serovars and *Chlamydia* species express the HMW
protein. *C. trachomatis* serovars B, Ba, D, F, G, I, J, K, L$_1$,
L$_2$, L$_3$, MoPn and *Chlamydia pneumoniae* were tested and found to
have a protein of an apparent molecular weight of 105-115 KDa
reactive with antisera generated against HMW protein.
25 **8.12. EXAMPLE 12: SURFACE LOCALIZATION**

Surface localization of the HMW protein on
different Chlamydia strains and derivatives were examined by
indirect fluorescence antibody (IFA). IFA was performed
using the procedures generally known in the art using
30 hyperimmune anti-HMW protein as the primary antibody. Hak
cells infected with whole EBs from one of *C. trachomatis*
serovars L$_2$, B, and F, *C. pneumoniae* or *C. psittaci* are
achieved by the following method.

McCoy or Hak cells were grown to confluence in D-
35 MEM media on 12 mm plain coverslips inside 24 well tissue
culture plates then centrifugally inoculated with ~5X10^4
inclusion forming units (IFU) of the *C. trachomatis* strain

N11 (serover F). After ~24 hours incubation,the culture
media was removed and infected cells fixed inmethanol for 10
min. The fixed monoloayer was then washed with PBS (1X) to
remove fixative and overlayer with >300 μl of anti-60Kdal
5 truncated HMWP rabbit antibody that had been diluted ~1/100
in PBS. After 1 hour incubation with the primary antibody,
the cells were washed gently with PBS then incubated for ~30
min. with a 1/200 dilution of a mouse anti-rabbit IgG
antibody conjugated with FITC. The second antibody was
10 diluted using a PBS solution containing 0.0091% Evans Blue as
a counter stain to visualize the monolayer. Cells were
washed 2X in PBS to remove the secondary antibody, the
coverslips removed from the culture plates, and mounted onto
microscope slides using a fluorescent mounting medium.
15   Identical cell samples stained with prebleed rabbit
antibody or FITC-conjugated second antibody alone were
processed in parallel and served as antibody specificity
(negative) controls. Counterstained samples were examined at
a 1000-X magnification with a Zeiss Axioskop photomicroscope
20 equipped with plan-neoflur objectives. Results using *C.
trachomatis* NI1 (F serovar) are shown in Figure 7. The
results show that enhanced fluorescence of samples stained
with HMW protein antibody compared to the controls confirmed
the surface location of the HMW protein. Furthermore,
25 fluorescence of samples stained with HMW protein antibodies
show binding to surface localized HMW protein from L_2, B and
MoPn serovars and *C. pneuomoniae*.

9.   **EXAMPLE: IN VITRO NEUTRALIZATION MODEL**
30   The *in vitro* neutralization model has been used to
show that protective antiserum inhibited chlamydial infection
(neutralization) of various tissue culture cell lines.
Animal models are also essential for testing vaccine efficacy
with both small animal (non-primate) and primate models
35 necessary for preclinical evaluation. The guinea-pig has
been used for studying experimental ocular and genital

infection by the Guinea-pig inclusion conjunctivitis agent
(GPIC), *C. psittaci*.

The mouse offers a consistent and reproducible
model of genital tract infection using human genital tract

5 isolates. This mouse model is a generally accepted pre-
clinical assay, and was used to evaluate MOMP as a subunit
vaccine. Another model is known as the primate model of
trachoma infection wherein the induction of secretory IgA was
shown to be a prime component of protection. Vaccinogenic

10 ability of new subunit antigen candidates is determined using
the above-mentioned generally accepted in vitro
neutralization and animal model systems.

As a preliminary exercise to the animal protection
studies, hyperimmune anti-HMW antibody was evaluated for its

15 ability to block the infectivity of various *C.trachomatis*
serovars (e.g. L_2,B,F) *in vitro*. Although McCoy cells were
used to propagate *Chlamydia*, these cells also allow antibody-
mediated uptake via Fc receptors. Therefore, to evaluate
anti-HMW antibody inhibition of infectivity, Hak cells, which

20 do not display Fc receptors, were used in these analyses.

Cells were grown on coverslips in 24-well plates to
a subconfluent monolayer (about 90% confluency = $1X10^5$
cells/ml) at 37°C in 5% CO_2. Anti-HMW-antibody was diluted to
about 100 μg/ml (total protein) in sucrose-phosphate-

25 glutamate (SPG) buffer and then serially diluted in SPG
buffer. Frozen aliquots of pretitered *Chlamydia* was diluted
in SPG buffer to about $2X10^4$ IFU/ml. EBs were premixed with
the diluted anti-HMW-antibody to about 10-20 IFU/μl and
incubated 30 minutes at 37°C on a rocking platform.

30 Prepared Hak cells were washed in HBSS and then
incubated with the anti-HMW-antibody/Chlamydia EB mixture in
triplicate for each antibody using 500 IFU/ml. Plates were
incubated for 2 hours at 37°C, then the inoculum removed and
plates washed 3 times with HBSS. Tissue culture media

35 containing 1 μg/ml of cyclohexamide was added and plates
incubated for about 24-36 hours at 37°C in 5% CO_2 to allow
inclusion bodies to develop. After incubation, the media was

removed and cell monolayers washed 3X in PBS. Plates were
fixed in methanol for 20 minutes and re-washed in PBS.

Cells were stained to visualize inclusions by
incubating with anti-*Chlamydia* LPS antibody (diluted about
5   1:500, ViroStat), cells washed 3 times in PBS, followed by
incubation with FITC-conjugated goat secondary antibody for
30 minutes at 37°C. Coverslips were washed, air dried, and
mounted in glycerol on glass coverslips. Inclusions were
counted in five fields through the midline of the coverslip
10 on a Zeiss fluorescence photomicroscope. Results are
reported as the percent reduction of inclusion-containing
cells with respect to a heterogenous antibody control (rabbit
prebleed sera).

15 **10. EXAMPLE: VACCINE EFFICACY (Mouse Model of Salpingitis
and Fertility)**

10.1. METHODS

10.1.1. IMMUNIZATION AND CHALLENGE

The Tuffrey murine infertility model was employed
20 to evaluate the efficacy of rHMWP to protect animals against
Chlamydia trachomatis-induced salpingitis and infertility.
Three groups of 17 female C3H HeOuJ mice (~6 weeks of age,
Jackson Labs) were employed for this evaluation. The test
group of 17 animals was immunized at weeks 0, 2, and 3 by
25 intranasal administration of ~20 μl of a vaccine formulation
containing approximately 10-12 μg of gel-purified rHMWP and
~5 μg mLT (SmithKline Beecham) as adjuvant. Prior to
immunization mice were sedated using an anesthesia cocktail
consisting of 16% Ketaject and 16% Xylaject in 68% pyrogen-
30 free PBS (100 μl i.p./animal). Sedated animals were placed
on their backs and using a standard laboratory pipette
administered the vaccine formulation; ~10 μl of the vaccine
solution per nostril with a 5-10 minute wait period between
applications.

35   Two groups of 17 female mice (per test group) were
immunized similarly but with a preparation containing only 5
μg mLT (*i.e.* adjuvant only, no antigen). One of these groups

was subsequently challenged with C.trachomatis (sham
immunized, infected) and served as the negative fertility
control while the other group was not challenged (sham
immunized, sham infected) and served as the positive

5   fertility control.

At week 4, all animals were administered a single
i.p. dose of progesterone (2.5 mg in pyrogen-free PBS, Depo-
Provera, Upjohn) to stabilize the uterine epithelium. At
week 5, animals immunized with rHMWP and animals in the

10   negative control group were infected by bilateral
intrauterine inoculation with ~5X10^5 inclusion forming units
(IFU) of C.trachomatis NI1 (serovar F) in 100 μl of sucrose
phosphate glutamate buffer (SPG). To mimic the manipulations
to the reproductive tract experienced by the other two

15   groups, animals in the positive control were bilaterally
inoculated with 100 μl of a McCoy cell extract that contained
no C.trachomatis. At week 7, 5-7 animals from each group
were sacrificed by CO_2 asphyxiation and the complete genital
tract (both upper and lower reproductive tracts) removed for

20   histopathological analysis. At week 9, the remaining females
from each group were caged with 8 - 10 week old male C3H mice
for a 2 month breeding period to assess fertility (1 male for
every 2 females per cage with weekly rotation of the males
within each group, animals from different experimental groups

25   were not mixed). Palpation and periodic weighing were used
to determine when animals in each pair became pregnant. The
parameters used to estimate group fertility were: F, the
number of mice which littered at least once during the mating
period divided by the total number of mice in that study

30   group; M, the number of newborn mice (born dead or alive)
divided by the number of litters produced in that group
during the mating period; and N, the number of newborn mice
(born dead or alive) divided by the total number of mice in
that group.

35   10.1.2.   HISTOPATHOLOGY

Genital tracts were treated for $\geq$ 24 hrs in Bouin's
fixative, progressively dehydrated in 50%, 70%, and 100%

methanol, soaked in toluol, and either paraffin embedded or
directly embedded in OCT compound (Tissue-TEK, Miles) and
subsequently snap frozen in liquid nitrogen. Tissue sections
(~6 μm) were stained with hematoxylin and eosin (after

5 deparaffinization of the Bouin fixed samples). Inflammatory
changes in the oviducts and ovaries were graded as follows:
0, no apparent inflammatory reaction; 1, a few mononuclear
cells infiltrating the periovarial space or the submucosa of
the oviduct; 2, same as 1 but to a greater extent; 3, same as

10 2 but with a thickened oviductal submucosa and the presence
of inflammatory cells in the oviductal lumen; 4, same as 3
but to a greater extent. Inflammation in the cervix/vagina
was scored based on the level of the intraepithelial
infiltrate observed.

15   10.1.3. DETERMINATION OF rHMWP-SPECIFIC HUMORAL
RESPONSES
           Blood samples were collected periodically during
the immunization and challenge phases by retroorbital
bleeding and serum prepared by centrifugation. Vaginal

20 secretions were collected by repeated injection of 50 μl of
sterile PBS into the vagina with a standard laboratory
pipettor and immediately withdrawing the solution. Two-to-
three injection/withdrawal cycles were performed.
           Quantitation of antibody (Ab) responses by ELISA

25 were performed as described in Section 8.11. Microwell ELISA
plates (Maxisorb, NUNC) for determining Ab levels were coated
overnight at 4°C with ~0.5-1.0 μg of gel-purified rHMWP per
well in 10mM carbonate/bicarbonate buffer (pH 9.6), washed
with PBS containing 0.1% Tween-20 (washing buffer) and

30 blocked for ~1hr at 37°C with a PBS solution containing 3%
BSA. For the determination of antigen-specific serum IgG
levels, test sera were serially diluted in washing buffer
containing 0.5% BSA and aliquots (100 μl) incubated in the
antigen-coated wells for ~2hr at 37°C. The plates were then

35 washed and incubated for ~1hr at 37°C with a horseradish
peroxidase (HRP)-conjugated goat anti-mouse IgG second
antibody (Sigma). A HRP-conjugated goat anti-mouse IgA

secondary antibody was used to detect the presence of rHMWP-specific IgA in vaginal secretions. After incubation with the appropriate secondary Ab, the plates were washed and incubated for ~20-30 minutes at room temperature with TMB

5 substrate (Sigma). Reactions were stopped by the addition of 2M H_2SO_4 and the absorbance determined at 450 nm on a Molecular Devices SpectroMax microplate reader. Titers were determined as the reciprocal of the sample dilution corresponding to an optical density of 1.0 at 450 nm.

10   10.1.4.   DETERMINATION OF rHMWP-SPECIFIC CELLULAR RESPONSES

          Groups of 6 female C3H HeOuJ mice (Jackson Labs) were sedated and immunized at weeks 0, 2, and 3 by intranasal administration with the rHMWP + mLT vaccine as described in

15 Section 10.1.3. At weeks 4 and 5 immediately prior to progesterone treatment and intrauterine challenge, respectively, 3 animals from each group were sacrificed by CO_2 asphyxiation and spleens aseptically removed and single cell suspensions prepared using conventional methodologies.

20 Spleen cells from immunized animals were analyzed separately. For both the positive control group (sham immunized and sham infected) and the negative control group (sham immunized, infected) spleen cells were pooled and tested for restimulation.

25   For the measurement of spleen cell proliferation, spleens were ground (5 to 10 rounds) in 5ml of RPMI 1640 Glutamax I supplemented with 10% fetal calf serum, 25 mM HEPES, 50 U/ml penicillin, 50 μg/ml streptomycin, 1 mm sodium pyruvate, nonessential amino acids, and 50 μM 2-

30 mercaptoethanol (Gibco-BRL). Live cells were counted by Trypan Blue staining and diluted in the same media to reach a density of 1.0 - $2.0X10^6$ cells/ml (Falcon 2063 polypropylene tubes). Triplicate cultures were set-up in round bottom 96-well culture plates (Nunclon, Nunc) using ~$5X10^5$ responder

35 cells per well in 200 μl of media Cells were stimulated with either 1.0μg/ml of rHMWP (antigen-specific proliferation) or with 4 μg/ml concanavalin A (Boerhinger Mannheim) as a

positive stimulation control; unrestimulated cell cultures
were used as a negative control of cellular activation.
After 72-96 hours of incubation at 37°C in 5% CO_2 cells were
pulsed labelled for ~18hrs with 1.0 μCi ^{3}H-thymidine
5 (Amersham) per well. Pulsed cells were harvested onto glass-
fiber sheets using a Tomtec Cell Harvester (Mk III) and
counted for beta-emission in a 3-channel Wallac 1450 Trilux
Liquid Scintillation Counter. The stimulation index (SI) for
a sample (individual or pooled) was defined as the mean of
10 the antigen or ConA-stimulated T-cell uptake of ^{3}H-thymidine
for triplicate wells divided by the mean of the unstimulated
uptake for triplicate wells. SIs for both antigen-specific
(rHMWP-specific) and ConA-specific proliferation were
determined.
15

        10.2. RESULTS
            10.2.1. EFFECT ON MOUSE FERTILITY AFTER A
HETEROTYPIC CHALLENGE
                Evidence that mucosal immunization with rHMWP
20 combined with mLT can afford protection against infertility
caused by a human clinical isolate of C.trachomatis (strain
NI1, serovar F) is shown in Table 1. Animals immunized with
the rHMWP displayed a significantly higher fertility rate
(70%, i.e. number of fertile females in group/total number of
25 animals in the group) than animals in the negative control
group (30%, sham immunized and infected). Similarly, the
rHMWP immunized group produced more offspring and exhibited a
group fecundity greater than those observed in the negative
control group (51 vs 24 offspring and 5.1 ± 4.7 vs 2.4 ±
30 4.6 fecundity scores, respectively). As a group, animals
immunized with the rHMWP vaccine displayed a comparable
fertility rate, total number of offspring, and a fecundity
score to those observed in the sham infected positive control
group (80% fertility rate, 56 total offspring, 4.9 ±2.7
35 fecundity).
                The protection against C.trachomatis-induced
infertility obtained in this experiment also demonstrates the

utility of the rHMWP to afford cross-biovar and cross-serovar protection against C.trachomatis disease. The recombinant HMWP antigen employed in this experiment was cloned from a strain belonging to the C.trachomatis lymphogranuloma
5 venereum (LGV) group (strain L2) which causes systemic as well as more localized mucosal infections of the eye and genital tract. The C.trachomatis challenge organism used in these experiments, strain NI1 is an F serovar organism that belongs to the trachoma biovar which causes numerous
10 urogenital tract infections.

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Table 1. Fertility Assessments Observed After ~2 Breeding
          Cycles

Group	Number of Animals per Group	Percent Fertile Animals	Number of Off-spring	Group Fecundity[1] (Mean + SD)
rHMWP-Immunized	10	70 p = 0.089[2]	51	5.10 $\pm$ 4.68 p = 0.105[3]
Sham Immunized Sham Infected (Positive Control)	10	80 p = 0.035	56	4.90 $\pm$ 2.70 p = 0.078
Sham Immunized Infected (Negative Control)	10	30	24	2.40 $\pm$ 4.61

[1]   Mean number of pups per group
[2]   Fisher's exact test, one-sided, 95% confidence interval
          p-values are given relative to the negative control

[3]   Student's t-test, unpaired, Gausian distribution, 95%
       confidence interval
          p-values are given relative to the negative control

10.2.2. EFFECT ON CELLULAR IMMUNE RESPONSE

The rHMWP-specific activation of the cellular
immune system was demonstrated using a conventional spleen
cell proliferation assay. When spleen cells were tested
during week 4 (immediately prior to progesterone treatment)
(Table 2) and week 5 (~7 days after hormone treatment but
before intrauterine challenge) (Table 3), all samples
collected from rHMWP-immunized animals developed a strong
antigen-specific proliferative immune response. The antigen-
specific Stimulation Indexes (SIs) obtained prior to
progesterone treatment from rHMWP-immunized animals were
equal to or greater than the SIs obtained via mitogenic
stimulation with ConA (mean values for antigen and ConA
stimulation obtained from 3 rHMWP-immunized animals: 26.2 vs
18.4, respectively). Spleen cells obtained from either sham

immunized animals or naive animals (i.e. animals that were not exposed to either the rHMWP antigen or mLT) did not respond to in vitro restimulation with the rHMWP material, thus establishing the specificity of the proliferative

5   response observed in the immunized animals. Progesterone treatment did not affect the antigen-specific proliferative response observed in rHMWP immunized animals. Antigen-specific SIs obtained with spleen cells obtained after hormone treatment were greater than obtained via mitogenic

10 stimulation (mean values for antigen and ConA stimulation obtained from 3 rHMWP-immunized animals: 92.4 vs 37.8, respectively). Again samples collected from sham immunized or naive animals failed to demonstrate any antigen-specific proliferative response.

15

Table 2.   rHMWP-Specific Cell Proliferation Before Hormone
           Treatment

Group	Cell Proliferation (cpm) Untreated / ConA / rHMWP	Stimulation Index (Treated cpm/ Untreated cpm) ConA / rHMWP
rHMWP Immunized Animal #1	1557 / 20739 / 65741	13.3 / 42.2
rHMWP Immunized Animal #2	1508 / 26975 / 28361	17.9 / 18.8
rHMWP Immunized Animal #3	1238 / 29991 / 23453	24.0 / 18.9
Sham-Immunized Animals (Pooled)	1687 / 30546 / 1292	18 / <1.0
Naive Animals (Pooled)	335 / 23886 / 838	71 / 2.5

35

Table 3. rHMWP-Specific Cell Proliferation After Hormone
          Treatment

Group	Cell Proliferation (cpm) Untreated / ConA / rHMWP	Stimulation Index (Treated cpm/ Untreated cpm) ConA / rHMWP
rHMWP Immunized Animal #1	767 / 15934 / 97458	20.8 / 127.0
rHMWP Immunized Animal #2	546 / 17212 / 28172	31.5 / 51.6
rHMWP Immunized Animal #3	297 / 18139 / 29300	61.1 / 98.6
Sham-Immunized Animal (Pooled)	273 / 18094 / 150	66.3 / <1.0
Naive Animals (Pooled)	345 / 16740 / 1341	48.5 / 3.9

10.2.3. EFFECT ON HUMORAL IMMUNE RESPONSE

To demonstrate that immunization with the full
length rHMWP produces a humoral immune response, IgG titers
were measured by ELISA on sera collected at week 5
immediately prior to challenge (i.e. approximately 2 weeks
after the third immunization). As shown in Table 4,
immunization of C3H mice with three doses of ~10-12 µg rHMWP
produced detectable levels of anti-rHMWP IgG in all animals.
Vaginal secretions were also collected from these animals at
the same time and tested for the presence of anti-rHMWP
mucosal IgA. Antigen-specific vaginal IgA was detected in
three animals (Table 4).

Table 4. rHMWP-Specific Humoral Response

rHMWP Immunized Animal	Anti-rHMWP Serum IgG ELISA Titer	Pres nc of Anti-rHMWP Vaginal IgA
4.4	5,000	
4.5	6,000	
4.6	12,000	+
4.7	130	
4.8	100	
4.9	54,000	+
4.10	670	
4.11	100	
4.12	570	
4.13	100,000	+
4.14	4,500	
4.15	400	
4.16	1,600	
4.17	2,500	
4.18	700	
4.19	70,000	
4.20	500	
4.21	2,000	
4.22	18,000	
4.23	3,000	
Mean ± S.D.	18.5 ± 29.6	

11. EXAMPLE: CONSTRUCTION OF PJJ701

A plasmid containing the entire *C.trachomatis* L_2 HMWP gene was constructed by selectively removing the EcoRI site upstream to the HMWP N-terminus in pAH306 (Described in Section 8.4). This was accomplished by digesting pAH306 to completion with XhoI and then religating the plasmid to

create pAH306-XhoΔ-1. The ~2.5 Kbp EcoRI fragment from
pAH316 (described in Section 8.5) which contains the
remaining HMWP C-terminus was isolated from preparative
agarose gels that had been loaded with a complete EcoRI
5 digestion of pAH316. An agarose gel slice containing the
appropriate ~2.5 Kbp fragment was then excised, dissolved
with NaI buffer and purified from residual agarose by
hydroxyapatite spin column chromatography (QiaGen). The
purified C-terminal HMWP EcoRI fragment was then ligated into
10 the single EcoRI site of pAH306-XhoΔ-1, located at the 3'-end
of the HMWP N-terminal coding sequence, using T4 DNA ligase
and standard molecular biology protocols. E.coli Top10 cells
were transformed with an aliquot of the pAH306-XhoΔ-1 (EcoRI
digested and phosphatase treated vector) and the 2.5 Kbp
15 EcoRI C-terminal fragment ligation reaction and recombinants
selected on 2X-YT agar containing 100 μg/ml ampicillin.
Ampicillin-resistant transformants were picked at random.
Plasmid DNA was isolated from individual Ap^R transformants
using a QiaGen Mini-Prep Plasmid DNA Isolation System and
20 screened for the presence of plasmids greater in size than
pAH306-XhoΔ-1 by conventional agarose gel electrophoresis and
ethidium bromide staining. Derivatives of pAH306-XhoΔ-1
carrying the 2.5 Kbp HMWP fragment in the proper orientation
that would allow expression of the full length HMWP were
25 identified by restriction analyses using EcoRI and/or XhoI.
Plasmid pAH374 was one derivative isolated from this
experiment.

A PCR-based site-directed mutagenesis procedure
(Quik-Change Site-Directed Mutagenesis System, Stratagene)
30 was employed to effect a desired DNA change, namely to remove
the NdeI site within the HMWP coding sequence of pAH374.
Mutagenic PCR primers, 41 bases in length and complementary
to the sequencing containing the NdeI site and designated
140-Nde-FX and 140-NdeRCX, were designed so as to eliminate
35 the NdeI recognition site but not change the corresponding
protein coding sequence. The sequences of the two PCR

mutagenic primers employed to remove the NdeI site in pAH374 are given below.

140-Nde-FX(SEQ ID NO: 38)

5 5'- GGG TTT GGG AAT CAG CAC ATG AAA ACC TCA TAT ACA TTT GC - 3'

140-Nde-RCX(SEQ ID NO:39)

5'- GCA AAT GTA TAT GAG GTT TTC ATG TGC TGA TTC CCA AAC CC - 10 3'

Following Pfu DNA polymerase (Stratagene) mutagenesis and DpnI digestion, to cleave any unaltered pAH374 parental plasmid, mutated plasmid DNA was then

15 transformed into E.coli XL1-Blue. Plasmid harboring transformants were selected on 2X-YT agar containing 100 μg/ml ampicillin. Antibiotic resistant transformants were picked at random and screened for plasmids of the size as pAH374. The identity of plasmids isolated from transformants

20 was determined by restriction enzyme digestion using EcoRI. The absence of the NdeI site in these plasmids was determined by digestion using NdeI. To verify the loss of the HMWP NdeI site and to ensure no unwanted DNA sequence changes had occurred in this region during the mutagenesis procedure,

25 mutagenized plasmids were further subjected to DNA sequence analysis using a sequence-specific sequencing primer located upstream of the NdeI site. Plasmid pAH374-NdeΔ-1 was one plasmid isolated from this experiment.

A DNA fragment encoding the C. trachomatis L$_2$ HMWP

30 without the internal NdeI site, plasmid pAH374-NdeΔ-1, was PCR amplified from reactions programmed with plasmid pAH374-NdeΔ-1 (~50 ng) and primers 306-Nde-Met1 and 312H6Xba1. Primer 306NdeMet1 was designed to contain a central NdeI site for directed cloning onto pMG81. The NdeI site in 306NdeMet1

35 overlapped the ATG start codon for HMWP signal sequence and was flanked by a 20 base G/C clamp on the 5' side and

- 76 -

sequences complementary to the first 15 residues of the HMWP
signal sequence on the 3' side.   Primer 312H6Xbal was
designed to contain sequences complementary to the C-terminus
of the HMWP followed by a (CAT)$_6$ motif specifying a hexa-

5   histidine affinity purification domain.   This primer also
contained two UAA termination codons, an XbaI recognition
sequence, and a 20 base G/C clamp at the 3' end of the
primer.   The sequences of the 306NdeMet1 and 312H6Xbal PCR
primers are given below.

10

306NdeMet1(SEQ ID NO: 40)
5'- AAG GGC CCA ATT ACG CAG ACA TAT GGA AAC GTC TTT CCA TAA
GTT CTT TCT TTC A - 3'

15 312H6Xbal(SEQ ID NO: 41)
5'- AAG GGC CCA ATT ACG CAG AGT CTA GAT TAT TAA TGA TGA TGA
TGA TGA TGG AAC CGG ACT CTA CTT CCT GCA CTC AAA CC - 3'

PCR amplification conditions described in Section
20 8.4 were used to generate the NdeI - XbaI HMWP gene cassette.
Following amplification, the PCR product was purified using
hydroxyapatite spin columns (QiaGen) and digested overnight
at 37°C with a ~10-fold excess of NdeI and XbaI to generate
the required 'overhangs' at the ends of the fragment.   The
25 digested fragment was again purified using spin columns and
~250 ng ligated to ~50 ng pMG81 plasmid DNA that had been
previously digested to completion with NdeI and XbaI and
subsequently treated with CIP to prevent vector religation.
An aliquot of the ligation reaction was used to transform
30 E.coli strain AR58 which had been made competent by the
method of Lederberg and Cohen.   Transformants were selected
on 2X-YT agar containing 40 μg/ml kanamycin sulfate.   Due to
the temperature inducible promoter on pMG81, the transformed
cells were grown at 30°C.   Kanamycin-resistant transformants
35 were picked at random and screened for the presence of
plasmids ~3.0 Kbp larger in size than pMG81.   Insert
containing derivatives of pMG81 were confirmed by restriction

enzyme analysis using NdeI, XbaI, EcoRI and NcoI. Plasmid pJJ701 was one plasmid isolated from this exercise.

12. Example 16: PRODUCTION OF FULL LENGTH rHMWP FROM AR58
5 (PJJ701)

One milliliter of a frozen stock of E. coli strain AR58 containing plasmid pJJ701 was used to inoculate ~100 ml of 2X-YT broth containing 40 μg/ml kanamycin and grown overnight at 30°C to prepare a fermentor seed culture.
10 Approximately 20 ml of the overnight seed culture was then used to inoculate a New Brunswick Bioflow 3000 fermentor loaded with ~2.0l of 2X-YT broth containing 40 μg/ml kanamycin. The AR58 (pJJ701) culture was grown at 30°C with vigorous aeration until an $O.D._{625}$ value of 0.5 - 0.6 was
15 attained. Expression of rHMWP was induced by increasing the temperature of the fermentor culture to ~39°C to 42°C. Incubation at the elevated temperature was continued for approximately 4 - 5 hours.

At the end of the induction period, the E. coli
20 culture, with some cells displaying classic recombinant protein inclusion bodies, was harvested by continuous flow centrifugation using an Heraeus Contifuge 28RS centrifuge. Following centrifugation, cell mass was scraped from the centrifuge bowl and stored at -70°C until processed.

25 Approximately 15 gm of the AR58 (pJJ701) frozen cell paste was resuspended by vortexing and trituration in ~40 ml of ice cold 10mM sodium phosphate buffer, pH7.3. Once suspended, lysozyme (Chicken egg white, Sigma) and DNase I (Bovine pancreas, Sigma) were added to final concentrations
30 of 1.0 mg/ml and 0.01 mg/ml, respectively, and the mixture incubated on ice for 30 - 45 minutes. Cells were disrupted by 2 sequential passes through a pre-cooled (~4°C) SLM Aminco French Pressure Cell (~14 Kpsi, 1" diameter bore). The cell lysate was then centrifuged for 5min at ~500Xg (4°C) in a
35 Sorvall SS34 rotor to remove unbroken cells. Insoluble material containing the rHMWP was isolated (pelleted) by centrifugation for 45min at ~20,000Xg (4°C) in a Sorvall SS34

rotor. The supernatant from this centrifugation was
discarded and the insoluble fraction stored at -20°C in pellet
form.

To selectively extract contaminating proteins and
5   remove endotoxin, the rHMWP-containing insoluble pellet was
thawed on ice and washed twice with 10ml of PBS buffer
containing 2.0% Triton X-100. Washing was performed at room
temperature and suspension of the gelatinous rHMWP-containing
pellet was accomplished by vortexing and homogenization in a
10 conventional glass tissue grinder. Insoluble material
containing the rHMWP was recovered after washing by
centrifugation at ~10,000Xg for 20 minutes (room temperature)
in a Sorvall SS34 rotor. Insoluble material was then washed
(again by vortexing and homogenization) 2-times with 10 ml of
15 a 4.0 M urea solution containing 2.0 M NaCl. Washed rHMWP
material was recovered by centrifugation as above. The
insoluble rHMWP fraction was further washed 2-times with 10
ml of a PBS solution containing 1.0% Zwittergent 3-14
(Sigma).

20   The rHMWP pellet recovered after centrifugation of
the final wash solution was then solubilized for 2 hours at
room temperature in standard Laemelli SDS-PAGE sample buffer
containing 4 M urea. Solubilized rHMWP was size fractionated
into a single protein band of ~110 Kdal by electrophoresis
25 through a standard ~14 cm X ~20 cm X ~3 mm 10% polyacrylamide
(36:1, acrylamide:bis-acrylamide) Tris/glycine/SDS
preparative gel. A 4% polyacrylamide stacking gel formed
using a 5-well, ~500 μl/well preparative comb was polymerized
on top of the resolving gel. Electrophoresis was carried out
30 on a BioRad Protean unit for ~12 hours at ~22°C (~80 - 85
volts, constant voltage) using a conventional
Tris/glycine/SDS running buffer (BioRad). Prestained
molecular weight standards (SeeBlue, Novex) were loaded into
a parallel lane and were used to gauge the degree and
35 efficiency of separation of the protein species. Following
electrophoresis, the gel sandwich was disassembled and a
vertical slice was removed from the rHMWP sample lane

adjacent to the molecular weight markers and stained with
coomassie blue R250 to visualize the rHMWP band. The stained
section was then repositioned onto the remaining unstained
preparative gel and the strip of acrylamide containing the
5 rHMWP identified and excised.

rHMWP was eluted from the gel slice using a
Schleicher and Schuell EluTrap electroelution device.
Electroelution was carried out according to the manufacturers
recommendations except 1/4-strength SDS running buffer
10 (Novex) was used as the elution buffer. Elution was carried
out at ~40 mA for ~12-14 hours, at room temperature. At the
end of the elution period the polarity of the cell was
reversed for ~2-3 minutes to remove any protein absorbed to
the BT1 membrane. The rHMWP-containing solution was removed
15 from the collection chamber and stored in a polypropylene
conical tube at 4°C.

Excess SDS detergent was removed using an SDS
precipitation system (SDS-OUT Precipitation kit, Pierce
Chemical). Removal of excess detergent from the gel-eluted
20 protein solution was accomplished following the
manufacturer's protocol. Detergent extracted rHMWP was
diluted approximately 15 fold with sterile, endotoxin-free
10m Molar sodium phosphate buffer (pH 7.4) and concentrated
to approximately 1.0 mg/ml by ultrafiltration in an Amicon
25 stirred concentration cell using a YM30 ultrafiltration
membrane.

Residual endotoxin was removed from the
concentrated rHMWP solution by polymyxin B Affi-Prep
Polymyxin Matrix (BioRad) treatment. Affi-Prep treatment was
30 performed overnight at 4°C in a batch mode according to the
manufacturers recommendations.

The protein concentration of the concentrated,
polymyxin B-treated rHMWP was determined using the Micro BCA
method (Pierce Chem.) and BSA as a standard.

35   Purified rHMWP (~0.9-1.2 mg/ml protein
concentration) was evaluated for purity, identity, and
residual endotoxin burden by SDS-PAGE, Western blot, and a

colorimetric endotoxin assay (BioWhittaker), respectively.
The gel-purified rHMWP material displayed a purity of >95% as
a single band of the expected molecular size (~110 Kdal) by
gel analysis and reacted vigorously with rHMWP-specific K196
5 antibody in Western blots. Residual endotoxin was calculated
to be $\leq$ 0.05 EU/μg.

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International Application No: PCT/

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page _39_, lines _20-21_ of the description '

A. IDENTIFICATION OF DEPOSIT '

Further deposits are identified on an additional sheet '

Name of depositary institution '

American Type Culture Collection

Address of depositary institution (including postal code and country) '

10801 University Blvd.
Manassas, VA 20110-2209
US

Date of deposit ' September 8, 1997 Accession Number ' 985538

B. ADDITIONAL INDICATIONS ' (leave blank if not applicable). This information is continued on a separate attached sheet

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE ' (if the indications are not all designated States)

D. SEPARATE FURNISHING OF INDICATIONS ' (leave blank if not applicable)

The Indications listed below will be submitted to the International Bureau later ' (Specify the general nature of the indications e.g., "Accession Number of Deposit")

E. ☑ This sheet was received with the International application when filed (to be checked by the receiving Office)

_____
(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau '

was
_____
(Authorized Officer)

Form PCT/RO/134 (January 1981)

WHAT IS CLAIMED IS:

1.   An isolated *Chlamydia* species HMW protein
wherein the apparent molecular weight is about 105-115 kDa,
5 as determined by SDS-PAGE, or a fragment or analogue thereof.

2.   The protein of claim 1 which is substantially
purified.

10   3.   The protein of claim 1 wherein the *Chlamydia*
species is *Chlamydia trachomatis, Chlamydia pecorum,
Chlamydia psittaci* or *Chlamydia pneumoniae*.

4.   The protein of claim 1 having an amino acid
15 sequence shown in SEQ ID No.: 2, 15 or 16 or a fragment or
conservatively substituted analogue thereof.

5.   The fragment of claim 4, having an amino acid
sequence shown in SEQ ID No.: 3, 17 or 25-37.
20

6.   The protein of claim 1 recognizable by an
antibody preparation that specifically binds to a peptide
having an amino acid sequence of SEQ ID No.: 2, 15 or 16 or a
fragment or conservatively substituted analogue thereof.
25

7.   An isolated nucleic acid molecule encoding the
HMW protein of claim 1 or a fragment or an analogue thereof.

8.   The nucleic acid molecule of claim 7 wherein
30 the *Chlamydia* species is *Chlamydia trachomatis, Chlamydia
pecorum, Chlamydia psittaci* or *Chlamydia pneumoniae*.

9.   The nucleic acid molecule of claim 7 wherein
the encoded protein has the amino acid sequence of SEQ ID No:
35 2, 15 or 16 or a fragment or conservatively substituted
analogue thereof.

10.   An isolated nucleic acid molecule having a
sequence selected from the group consisting of:

    a)   a DNA sequence of SEQ ID No.: 1, 23 or 24, or
          a complementary sequence or fragment thereof;

    b)   a DNA sequence encoding a HMW protein having
          the amino acid sequence of SEQ ID No.: 2, 15
          or 16 or fragment thereof;

    c)   a DNA sequence encoding a deduced amino acid
          sequence of SEQ ID No.: 2, 15 or 16 or the
          complimentary or degenerate sequence thereto
          or fragment thereof; and

    d)   a nucleic acid sequence which hybridizes under
          stringent conditions to any one of the
          sequences defined in a), b) or c).

11.   A recombinant expression vector adapted for
transformation of a host comprising the nucleic acid molecule
of claim 7 or 10.

12.   A recombinant expression vector adapted for
transformation of a host comprising the nucleic acid molecule
of claim 7 or 10 and expression means operatively coupled to
the nucleic acid molecule for expression by the host of HMW
protein or a fragment or analogue thereof.

13.   The expression vector of claim 12, wherein the
expression means includes a nucleic acid portion encoding a
leader sequence for secretion from the host of the HMW
protein or a fragment or analogue thereof.

14.   A transformed host cell containing an
expression vector of claim 12.

15.   A transformed host cell containing an
expression vector of claim 13.

16. An isolated recombinant protein or fragment or
analogue thereof producible by the transformed host of claim
14.

5   17. An isolated recombinant protein or fragment or
analogue thereof producible by the transformed host of claim
15.

           18. A recombinant vector for delivery of a HMW
10 protein or fragment or analogue thereof to a host comprising
the nucleic acid molecule of claim 7 or 10.

           19. An immunogenic composition, comprising at
least one component selected from the group consisting of:
15   a)   an isolated HMW protein, wherein the apparent
                molecular weight is about 105-115 kDa, as
                determined by SDS-PAGE, or a fragment or
                conservatively substituted analogue thereof;
           b)   an isolated nucleic acid molecule encoding a
20   HMW protein of a) or a fragment or analogue
                thereof;
           c)   an isolated nucleic acid molecule having the
                sequence of SEQ ID No.: 1, 23 or 24, the
                complimentary sequence thereto or a nucleic
25   acid sequence which hybridizes under stringent
                conditions thereto or fragment thereof;
           d)   an isolated recombinant protein or fragment or
                analogue thereof producible in a transformed
                host comprising an expression vector
30   comprising a nucleic acid molecule as defined
                in b) or c) and expression means operatively
                coupled to the nucleic acid molecule for
                expression by the host of said HMW protein or
                the fragment or analogue thereof;
35   e)   a recombinant vector comprising a nucleic acid
                sequence of b) or c) encoding a HMW protein or
                fragment or analogue thereof; and

f)   a transformed cell comprising the vector of
      e),

and optionally an adjuvant, and a pharmaceutically acceptable
carrier or diluent therefor, said composition producing an
5 immune response when administered to a host.

      20.   An antigenic composition, comprising at least
one component selected from the group consisting of:
      a)   an isolated HMW protein, wherein the apparent
10   molecular weight is about 105-115 kDa as
            determined by SDS-PAGE, or a fragment or
            analogue thereof;
      b)   an isolated nucleic acid molecule encoding a
            HMW protein of a), or a fragment or analogue
15   thereof;
      c)   an isolated nucleic acid molecule having the
            sequence of SEQ ID No.: 1, 22, 23 or 24, the
            complimentary or degenerate sequence thereto
            or a nucleic acid sequence which hybridizes
20   under stringent conditions thereto;
      d)   an isolated recombinant protein or fragment or
            analogue thereof producible in a transformed
            host comprising an expression vector
            comprising a nucleic acid molecule as defined
25   in b) or C) and expression means operatively
            coupled to the nucleic acid molecule for
            expression by the host of said HMW protein or
            the fragment or analogue thereof;
      e)   a recombinant vector, comprising a nucleic
30   acid sequence of b) or c) encoding a HMW
            protein or fragment or analogue thereof; and
      f)   a transformed cell comprising the vector of
            e),

and optionally an adjuvant, said composition producing an
35 immune response when administered to a host.

21.   A method of producing an immune response in an
animal comprising administering to said animal an effective
amount of the antigenic composition of claim 20 or the
immunogenic composition of claim 19.

5

22.   The method of claim 21 wherein the animal is a
mammal or a bird.

23.   Antisera raised against the antigenic
10 composition of claim 20 or the immunogenic composition of
claim 19.

24.   Antibodies present in the antisera of claim 23
that specifically bind a HMW protein or a fragment or
15 analogue thereof.

25.   A diagnostic reagent selected from the group
consisting of: the protein of claim 1, the nucleic acid
molecule of claim 10, the immunogenic composition of claim
20 20, the antigenic composition of claim 19, the antisera of
claim 23, the vector of claim 12, the transformed cell of
claim 14, and the antibodies of claim 24.

26.   A method for detecting anti-*Chlamydia*
25 antibodies in a test sample comprising the steps of:
      a)   contacting a sample with the HMW protein of
           claim 1, the antigenic composition of claim 20
           or the immunogenic composition of claim 19 to
           form, in the presence of said antibodies,
30   *Chlamydia* antigen: anti-*Chlamydia* antibody
           immunocomplexes, and further,
      b)   either detecting the presence of or measuring
           the amount of said immunocomplexes formed
           during step a) as an indication of the
35   presence of said anti-*Chlamydia* antibodies in
           the test sample.

27. A diagnostic kit for detecting antibodies to
Chlamydia, said kit comprising the HMW protein of claim 1,
the antigenic composition of claim 20 or the immunogenic
composition of claim 19, a container means for contacting
5 said protein or composition with a test sample suspected of
having said antibodies and reagent means for detecting or
measuring *Chlamydia* antigen: anti-*Chlamydia* antibody
immunocomplexes formed between said protein or composition
and said antibodies.
10

28. A method for detecting the presence of
Chlamydia in a test sample comprising the steps of:
    a)   contacting a test sample with the antibodies
          of claim 24 for a time sufficient to allow
15   said antibodies to bind *Chlamydia*, if present,
          and to form a *Chlamydia*: anti-*Chlamydia*
          antibody immunocomplexes, and further,
    b)   either detecting the presence of or measuring
          the amount of said immunocomplexes formed
20   during step a) as an indication of the
          presence of said *Chlamydia* in the test sample.

29. A diagnostic kit for detecting the presence of
Chlamydia, said kit comprising the antibodies of claim 24,
25 container means for contacting said antibodies with a test
sample suspected of having said *Chlamydia* and reagent means
for detecting or measuring *Chlamydia*: anti-*Chlamydia* antibody
immunocomplexes formed between said antibodies and said
Chlamydia.
30

30. A pharmaceutical composition comprising an
effective amount of at least one component selected from the
group consisting of:
    a)   a HMW protein, wherein the apparent molecular
35   weight is about 105-115 kDa, as determined by
          SDS-PAGE, or a fragment or analogue thereof;

b)   an isolated nucleic acid molecule encoding a
       HMW protein of a), or a fragment or analogue
       thereof;

c)   an isolated nucleic acid molecule having the

5   sequence of SEQ ID No.: 1, 23 or 24, the
       complimentary or degenerate sequence thereto
       or a nucleic acid sequence which hybridizes
       under stringent conditions thereto;

d)   an isolated recombinant HMW protein or

10   fragment or analogue thereof producible in a
       transformed host comprising an expression
       vector comprising a nucleic acid molecule as
       defined in b) or c) and expression means
       operatively coupled to the nucleic acid

15   molecule for expression by the host of said
       HMW protein or the fragment or analogue
       thereof;

e)   a recombinant vector, comprising a nucleic
       acid sequence of b) or c) encoding a HMW

20   protein or fragment or analogue thereof;

f)   a transformed cell comprising the vector of
       e), and

g)   antibodies that specifically bind the
       component of a), b), c), d), e) or f),

25 and optionally a pharmaceutically acceptable carrier or
diluent therefor.

       31.   A vaccine composition comprising an effective
amount of at least one component selected from the group
30 consisting of:

a)   a HMW protein, wherein the apparent molecular
       weight is about 105-115 kDa, as determined by
       SDS-PAGE, or a fragment or analogue thereof;

b)   an isolated nucleic acid molecule encoding a

35   HMW protein of a) or a fragment or analogue
       thereof;

c)   an isolated nucleic acid molecule having the
        sequence of SEQ ID No.: 1, 23 or 24, the
        complimentary or degenerate sequence thereto
        or a nucleic acid sequence which hybridizes
5   under stringent conditions thereto;

d)   an isolated recombinant HMW protein or
        fragment or analogue thereof producible in a
        transformed host comprising an expression
        vector comprising a nucleic acid molecule as
10   defined in b) or c) and expression means
        operatively coupled to the nucleic acid
        molecule for expression by the host of said
        HMW protein or the fragment or analogue
        thereof;

15 e)   a recombinant vector, comprising a nucleic
        acid sequence of b) or c) encoding a HMW
        protein or fragment or analogue thereof;

f)   a transformed cell comprising the vector of
        e), and

20 g)   antibodies that specifically bind the
        component of a), b), c), d), e), or f),
    and optionally an adjuvant, and a pharmaceutically acceptable
    carrier or diluent therefor, wherein the vaccine produces an
    immune response when administered to a host.
25

        32.   A method of preventing, treating or
ameliorating a disorder related to *Chlamydia* in a host in
need of such treatment comprising administering to a host, an
effective amount of the pharmaceutical composition of claim
30 30 or the vaccine composition of claim 31.

        33.   The method of claim 32, wherein the disorder
is selected from the group consisting of a *Chlamydia*
bacterial infection, conjunctivitis, urethritis,
35 lymphogranuloma venereum (LGV), cervicitis, epididymitis,
endometritis, pelvic inflammatory disease (PID), salpingitis,

tubal occlusion, infertility, cervical cancer,
arteriosclerosis and atherosclerosis.

34.   The method of claim 33 wherein the host is a
5 bird or a mammal.

35.   The composition of any one of claims 19, 20,
30 or 31 formulated for *in vivo* administration to a host to
confer protection against disease caused by a species of
10 *Chlamydia*.

36.   The composition of any one of claims 19, 20,
30, or 31 wherein the species is selected from the group
consisting of *Chlamydia trachomatis*, *Chlamydia pecorum*,
15 *Chlamydia psittaci* and *Chlamydia pneumoniae*.

37.   The composition of any one of claims 19, 20,
30 or 31 formulated as a microparticle, capsule, or liposome
preparation.
20

38.   The protein of any one of claims 1, 4, 6, 16
and 17, wherein the protein binds to heparin or heparan-
sulfate.

25   39.   The protein of any one of claims 1, 4, 6, 16
or 17, wherein the protein is an outer membrane protein.

40.   A method for determining the presence of
nucleic acid encoding a HMW protein or a fragment or analogue
30 thereof in a sample, comprising the steps of:
        a)   contacting a sample with the nucleic acid
              molecule of claim 7 or 10 or any fragment
              thereof or complementary thereto to produce
              duplexes comprising the nucleic acid molecule
35   and any said nucleic acid molecule encoding
              the HMW protein in the sample and specifically
              hybridizable therewith; and

b)   determining the production of duplexes.

41.   A diagnostic kit for determining the presence
of nucleic acid encoding a HMW protein or fragment or
5 analogue thereof in a sample, comprising:

a)   the nucleic acid molecule of claim 7 or 10 or
any fragment thereof or complementary thereto;

b)   means for contacting the nucleic acid with the
sample to produce duplexes comprising the
10   nucleic acid molecule and any said nucleic
acid encoding the HMW protein in the sample
and specifically hybridizable therewith; and

c)   means for determining the production of
duplexes.
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Figure 1

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98 —
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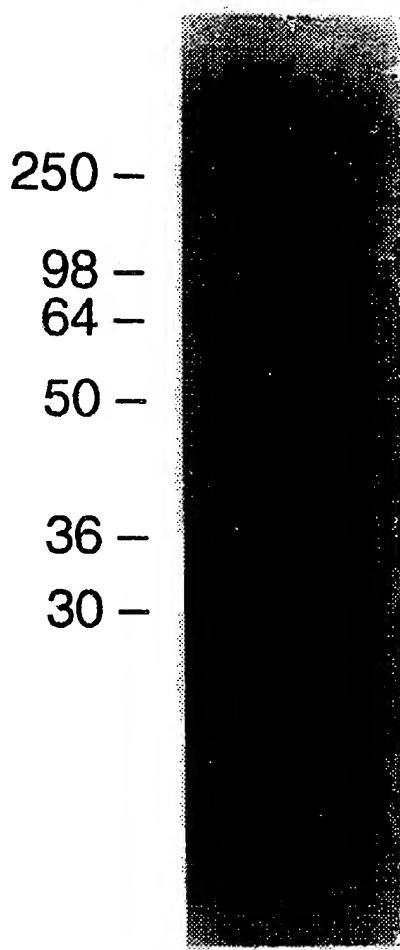
GGGCAAAACTCTTCCCCCCGGGATTTATATGGGAAAGGGGAAACTTTGGC

CCGTATTCAAGCGCCACGGGTTTTGGGGCGGAATGAATTTTTTCGTTCCG

GAAAAAGTAATTCCCCGGGAACGTAGGGTATCGGTTTCATAGGCTCGCCA

AATGGGATATAGGTGGAAAGGTAAAAAAAACTGAGCCAAGCAAAGGATAG

AGAAGTCTTGTAATCATCGCAGGTTAAAGGGGGGATGTTATTTTAGCCTG

CAAATAGTGTAATTATTGGATCCTGTAAAGAGAAAAGGACGAATGCGCTG

AAGATAAGAACATTTATTGATATTAAATTATTAATTTTTTATGAAGCGGA

GTAATTAATTTTATCTCTCAGCTTTTGTGTGATGCAAACGTCTTTCCATA

AGTTCTTTCTTTCAATGATTCTAGCTTATTCTTGCTGCTCTTTAAATGGG

GGGGGATATGCAGCAGAAATCATGGTTCCTCAAGGAATTTACGATGGGGA

GACGTTAACTGTATCATTTCCCTATACTGTTATAGGAGATCCGAGTGGGA

CTACTGTTTTTTCTGCAGGAGAGTTAACATTAAAAAATCTTGACAATTCT

ATTGCAGCTTTGCCTTTAAGTTGTTTTGGGAACTTATTAGGGAGTTTTAC

TGTTTTAGGGAGAGGACACTCGTTGACTTTCGAGAACATACGGACTTCTA

CAAATGGGGCAGCTCTAAGTAATAGCGCTGCTGATGGACTGTTTACTATT

GAGGGTTTTAAAGAATTATCCTTTTCCAATTGCAATTCATTACTTGCCGT

ACTGCCTGCTGCAACGACTAATAAGGGTAGCCAGACTCCGACGACAACAT

CTACACCGTCTAATGGTACTATTTATTCTAAAACAGATCTTTTGTTACTC

AATAATGAGAAGTTCTCATTCTATAGTAATTTAGTCTCTGGAGATGGGGG

AGCTATAGATGCTAAGAGCTTAACGGTTCAAGGAATTAGCAAGCTTTGTG

TCTTCCAAGAAAATACTGCTCAAGCTGATGGGGGGAGCTTGTCAAGTAGTC

ACCAGTTTCTCTGCTATGGCTAACGAGGCTCCTATTGCCTTTGTAGCGAA

TGTTGCAGGAGTAAGAGGGGGAGGGATTGCTGCTGTTCAGGATGGGCAGC

AGGGAGTGTCATCATCTACTTCAACAGAAGATCCAGTAGTAAGTTTTTCC

AGAAATACTGCGGTAGAGTTTGATGGGAACGTAGCCCGAGTAGGAGGAGG

GATTTACTCCTACGGGAACGTTGCTTTCCTGAATAATGGAAAAACCTTGT

TTCTCAACAATGTTGCTTCTCCTGTTTACATTGCTGCTAAGCAACCAACA

AGTGGACAGGCTTCTAATACGAGTAATAATTACGGAGATGGAGGAGCTAT

CTTCTCTAAGAATGGTGCGCAAGCAGGATCCAATAACTCTGGATCAGTTT

CCTTTGATGGAGAGGGAGTAGTTTTCTTTAGTAGCAATGTAGCTGCTGGG

AAAGGGGGAGCTATTTATGCCAAAAAGCTCTCGGTTGCTAACTGTGGCCC

TGTACAATTTTTAAGGAATATCGCTAATGATGGTGGAGCGATTTATTTAG

GAGAATCTGGAGAGCTCAGTTTATCTGCTGATTATGGAGATATTATTTTC

FIG. 2A

GATGGGAATCTTAAAAGAACAGCCAAAGAGAATGCTGCCGATGTTAATGG

CGTAACTGTGTCCTCACAAGCCATTTCGATGGGATCGGGAGGGAAAATAA

CGACATTAAGAGCTAAAGCAGGGCATCAGATTCTCTTTAATGATCCCATC

GAGATGGCAAACGGAAATAACCAGCCAGCGGCAGTCTTCCAAACTTCTAAA

AATTAACGATGGTGAAGGATACACAGGGGATATTGTTTTTGCTAATGGAA

GCAGTACTTTGTACCAAAATGTTACGATAGAGCAAGGAAGGATTGTTCTT

CGTGAAAAGGCAAAATTATCAGTGAATTCTCTAAGTCAGACAGGTGGGAG

TCTGTATATGGAAGCTGGGAGTACATGGGATTTTGTAACTCCACAACCAC

CACAACAGCCTCCTGCCGCTAATCAGTTGATCACGCTTTCCAATCTGCAT

TTGTCTCTTTCTTCTTTGTTAGCAAACAATGCAGTTACGAATCCTCCTAC

CAATCCTCCAGCGCAAGATTCTCATCCTGCAGTCATTGGTAGCACAACTG

CTGGTTCTGTTACAATTAGTGGGCCTATCTTTTTTGAGGATTTGGATGAT

ACAGCTTATGATAGGTATGATTGGCTAGGTTCTAATCAAAAAATCAATGT

CCTGAAATTACAGTTAGGGACTAAGCCCCCAGCTAATGCCCCATCAGATT

TGACTCTAGGGAATGAGATGCCTAAGTATGGCTATCAAGGAAGCTGGAAG

CTTGCGTGGGATCCTAATACAGCAAATAATGGTCCTTATACTCTGAAAGC

TACATGGACTAAAACTGGGTATAATCCTGGGCCTGAGCGAGTAGCTTCTT

TGGTTCCAAATAGTTTATGGGGATCCATTTTAGATATACGATCTGCGCAT

TCAGCAATTCAAGCAAGTGTGGATGGGCGCTCTTATTGTCGAGGATTATG

GGTTTCTGGAGTTTCGAATTTCTTCTATCATGACCGCGATGCTTTAGGTC

AGGGATATCGGTATATTAGTGGGGGGTTATTCCTTAGGAGCAAACTCCTAC

TTTGGATCATCGATGTTTGGTCTAGCATTTACCGAAGTATTTGGTAGATC

TAAAGATTATGTAGTGTGTCGTTCCAATCATCATGCTTGCATAGGATCCG

TTTATCTATCTACCCAACAAGCTTTATGTGGATCCTATTTGTTCGGAGAT

GCGTTTATCCGTGCTAGCTACGGGTTTGGGAATCAGCATATGAAAACCTC

ATATACATTTGCAGAGGAGAGCGATGTTCGTTGGGATAATAACTGTCTGG

CTGGAGAGATTGGAGCGGGATTACCGATTGTGATTACTCCATCTAAGCTC

TATTTGAATGAGTTGCGTCCTTTCGTGCAAGCTGAGTTTTCTTATGCCGA

TCATGAATCTTTTACAGAGGAAGGCGATCAAGCTCGGGCATTCAAGAGCG

GACATCTCCTAAATCTATCAGTTCCTGTTGGAGTGAAGTTTGATCGATGT

TCTAGTACACATCCTAATAAATATAGCTTTATGGCGGCTTATATCTGTGA

TGCTTATCGCACCATCTCTGGTACTGAGACAACGCTCCTATCCCATCAAG

AGACATGGACAACAGATGCCTTTCATTTAGCAAGACATGGAGTTGTGGTT

AGAGGATCTATGTATGCTTCTCTAACAAGTAATATAGAAGTATATGGCCA

TGGAAGATATGAGTATCGAGATGCTTCTCGAGGCTATGGTTTGAGTGCAG

FIG. 2B

GAAGTAGAGTCCGGTTCTAAAAATATTGGTTAGATAGTTAAGTGTTAGCG
ATGCCTTTTTCTTTGAGATCTACATCATTTTGTTTTTTAGCTTGTTTGTG
TTCCTATTCGTATGGATTCGCGAGCTCTCCTCAAGTGTTAACGCCTAATG
TAACCACTCCTTTTAAGGGAGACGATGTTTACTTGAATGGAGACTGCGCT
TTTGTCAATGTCTATGCAGGAGCTGAAGAAGGTTCGATTATCTCAGCTAA
TGGCGACAATTTAACGATTACCGGACAAAACCATACATTATCATTTACAG
ATTCTCAAGGGCCAGTTCTTCAAAATTATGCCTTCATTTCAGCAGGAGAG
ACACTTACTCTGAGAGATTTTTCGAGTCTGATGTTCTCGAAAAATGTTTC
TTGCGGAGAAAAGGGAATGATCTCCGGGAAAACCGTGAGTATTTCCGGAG
CAGGCGAAGTGATTTTCTGGGATAACTCCGTGGGGTATTCTCCTTTATCT
ACTGTGCCAACCTCATCATCAACTCCGCCTGCTCCAACAGTTAGTGATGC
TCGGAAAGGGTCTATTTTTTCTGTAGAGACTAGTTTGGAGATCTCAGGCG
TCAAAAAAGGGGTCATGTTCGATAATAATGCCGGGAATTTCGGAACAGTT
TTTCGAGGTAAGAATAATAATAATGCTGGTGGTGGAGGCAGTGGGTTCCG
CTACACCATCAAGTACGACTTTTACAGTTAAAAACTGTAAAGGGAAAGTT
TCTTTCACAGATAACGTAGCCTCTTGCGGAGGCGGAGTGGTTTATAAAGG
CATTGTGCTTTTCAAAGACAATGAAGGAGGCATATTCTTCCGAGGGAACA
CAGCATACGATGATTTAAGGATTCTTGCTGCTACTAATCAGGATCAGAAT
ACGGAGACAGGAGGCCGGTGGAGGAGTTATTTGCTCTCCAGATGATTCTGT
AAAGTTTGAAGGCAATAAAGGTTCTATTGTTTTTGATTACAACTTTGCAA
AAGGCAGAGGCGGAAGCATCCTAACGAAAGAATTC

FIG. 2C

MQTSFHKFFLSMILAYSCCSLNGGGYAAEIMVPQGIYDGETLTVSFPYTV

IGDPSGTTVFSAGELTLKNLDNSIAALPLSCFGNLLGSFTVLGRGHSLTF

ENIRTSTNGAALSNSAADGLFTIEGFKELSFSNCNSLLAVLPAATTNKGS

QTPTTTSTPSNGTIYSKTDLLLLNNEKFSFYSNLVSGDGGAIDAKSLTVQ

GISKLCVFQENTAQADGGACQVVTSFSAMANEAPIAFVANVAGVRGGGIA

AVQDGQQGVSSSTSTEDPVVSFSRNTAVEFDGNVARVGGGIYSYGNVAFL

NNGKTLFLNNVASPVYIAAKQPTSGQASNTSNNYGDGGAIFCKNGAQAGS

NNSGSVSFDGEGVVFFSSNVAAGKGGAIYAKKLSVANCGPVQFLRNIAND

GGAIYLGESGELSLSADYGDIIFDGNLKRTAKENAADVNGVTVSSQAISM

GSGGKITTLRAKAGHQILFNDPIEMANGNNQPAQSSKLLKINDGEGYTGD

IVFANGSSTLYQNVTIEQGRIVLREKAKLSVNSLSQTGGSLYMEAGSTWD

FVTPQPPQQPPAANQLITLSNLHLSLSSLLANNAVTNPPTNPPAQDSHPA

VIGSTTAGSVTISGPIFFEDLDDTAYDRYDWLGSNQKINVLKLQLGTKPP

ANAPSDLTLGNEMPKYGYQGSWKLAWDPNTANNGPYTLKATWTKTGYNPG

PERVASLVPNSLWGSILDIRSAHSAIQASVDGRSYCRGLWVSGVSNFFYH

DRDALGQGYRYISGGYSLGANSYFGSSMFGLAFTEVFGRSKDYVVCRSNH

HACIGSVYLSTQQALCGSYLFGDAFIRASYGFGNQHMKTSYTFAEESDVR

WDNNCLAGEIGAGLPIVITPSKLYLNELRPFVQAEFSYADHESFTEEGDQ

ARAFKSGHLLNLSVPVGVKFDRCSSTHPNKYSFMAAYICDAYRTISGTET

TLLSHQETWTTDAFHLARHGVVVRGSMYASLTSNIEVYGHGRYEYRDASR

GYGLSAGSRVRF

FIG.3
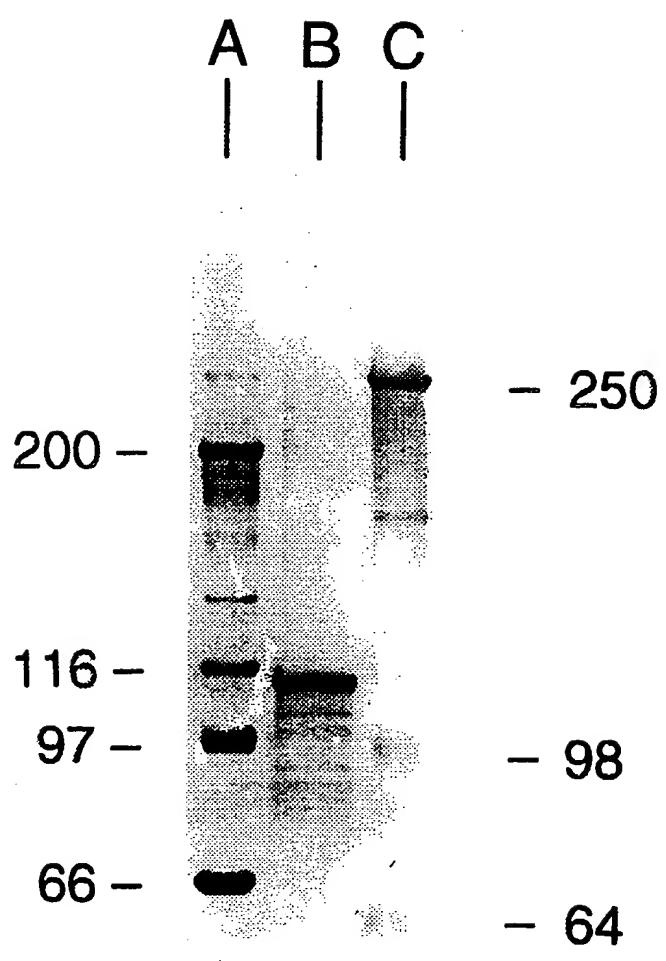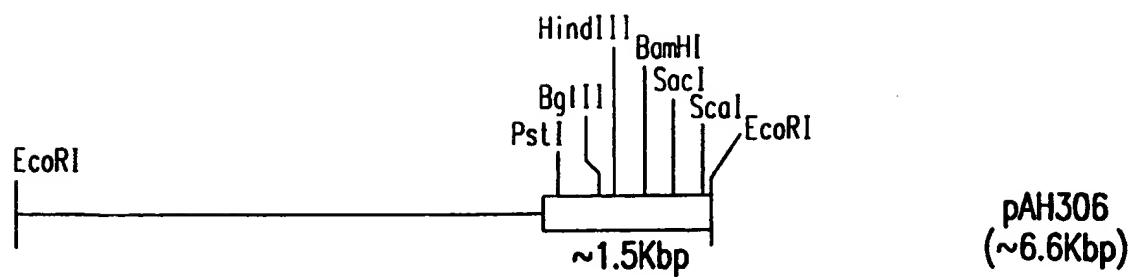
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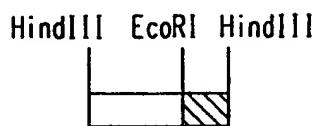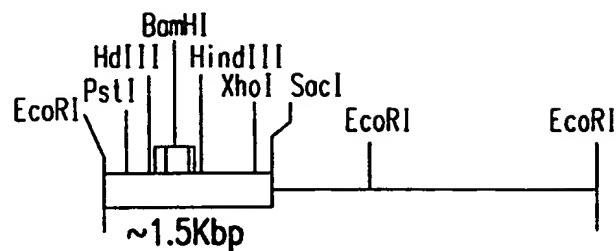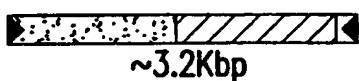
FIG.5

```
L2                                        E IMVPQGIYDGETLTVSFPYTVIGDPSGTTVF
F
B


                                          100*
L2   SAGELTLKNLDNSIAALPLSCFGNLLGSFTVLGRCHSLTFENIRTSTNGAALSNSAADGL
F                                                              D  NS
B                                                              D  NS


L2   FTIEGFKELSFSNCNSLLAVLPAATTNKGSQTPTTTSTPSNGTIYSKTDLLLLNNEKFSFY
F                     |                N       |
B                     P                N       S

     200*
L2   SNLVSGDGGAIDAKSLTVQGISKLCVFQENTAQADGGACQVVTSFSAMANEAPIAFVA
F         |          T                                       |
B         S


L2   NVAGVRGGGIAAVQDGQQGVSSSTSTEDPVVSFSRNTAVEFDGNVARVGGGIYSYGNV


     *300
L2   AFLNNGKTLFLNNVASPVYIAAKQPTSGQASNTSNNYGDGGAIFCKNGAQAGSNNSGS
F                          E     N        D            [A]
B                          E     N        D            [A]


                                        400*
L2   VSFDGEGVVFFSSNVAAGKGGAIYAKKLSVANCGPVQFLRNIANDGGAIYLGESGELSL
F                                                |  G
B                                                L  G


L2   SADYGDIIFDGNLKRTAKENAADVNGVTVSSQAISMGSGGKITTLRAKAGHQILFNDPIE
F              |
B              M

                             500*                    EcoRI- I
L2   MANGNNQPAQSSKLLKINDGEGYTGDIVFANGSSTLYQNVTIEQGRIVLREKAKLSVNSL
F                     EP                  N
B                     EP                  N
```

FIG. 6A

L2   SQTGGSLYMEAGSTWDFVTPQPPQQPPAANQLITLSNLHLSLSSLLANNAVINPPTNP
F   V
B   L   S

      600*
L2   PAQDSHPAVIGSTTAGSVTISGPIFFEDLDDTAYDRYDWLGSNQKINVLKLQLGTKPPA
F   P   F   D   Q S
B   D   Q S

                                 700*
L2   NAPSDLTLGNEMPKYGYQGSWKLAWDPNTANNGPYTLKATWTKTGYNPGPERVASLV
F
B

L2   PNSLWGSILDIRSAHSAIQASVDGRSYCRGLWVSGVSNFFYHDRDALGQGYRYISGGYS
F   S
B

                        800*
L2   LGANSYFGSSMFGLAFTEVFGRSKDYVVCRSNHHACIGSVYLSTQQALCGSYLFGDAFI
F   K
B   K   V

L2   RASYGFGNQHMKTSYTFAEESDVRWDNNCLAGEIGAGLPIVITPSKLYLNELRPFVQAEF
F   V   V   T
B   C   V   V

      900*
L2   SYADHESFTEEGDQARAFKSGHLLNLSVPVGVKFDRCSSTHPNKYSFMAAYICDAYRTI
F   R   M   G
B   R   M   G

                                          1000*
L2   SGTETTLLSHQETWTTDAFHLARHGVVVRGSMYASLTSNIEVYGHGRYEYRDASRCYGL
F   Q   I   T
B   Q   I   T

      1013*
L2   SAGSRVRF
F   K
B   K

FIG. 6B

Figure 7A

Figure 7 B
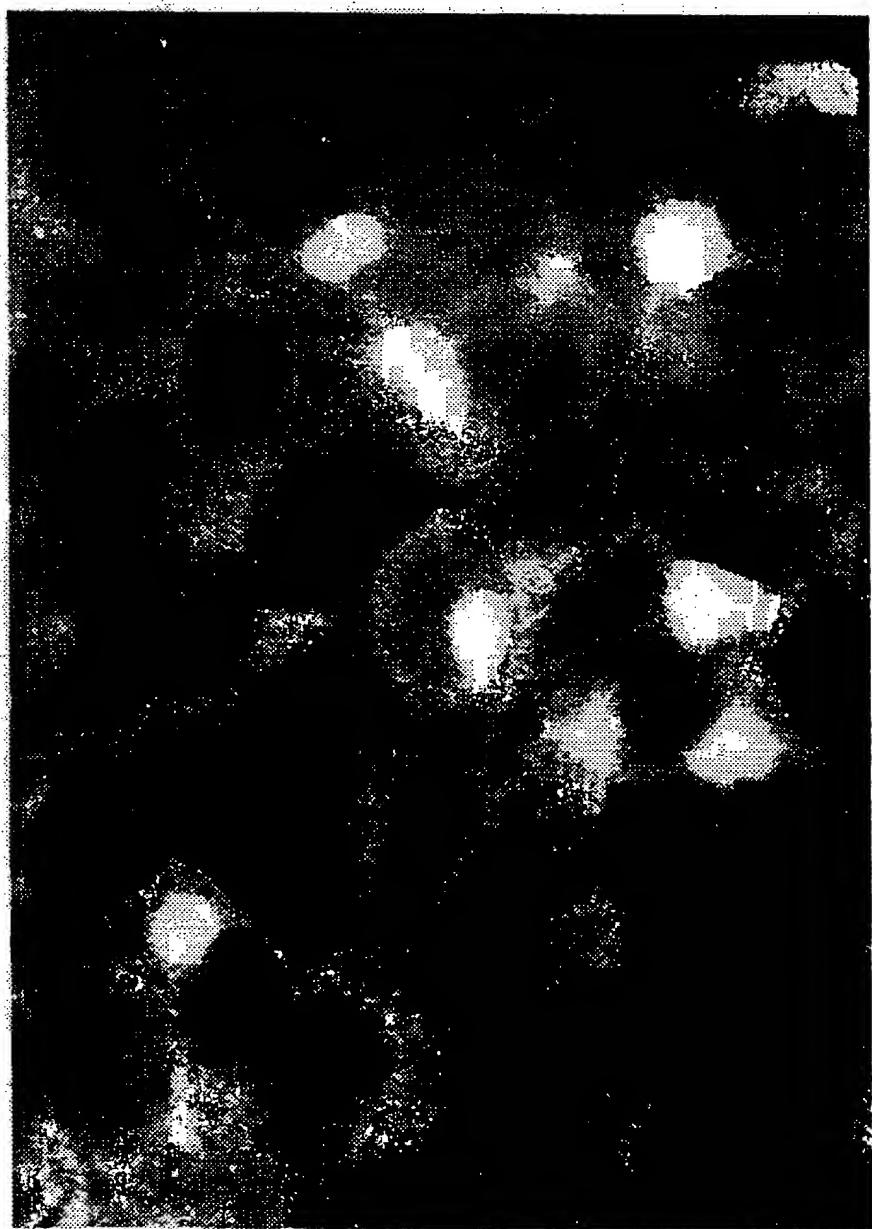
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<120> CHLAMYDIA PROTEIN, GENE SEQUENCE AND USES THEREOF

<130> 7969-076

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<151> 1997-10-02

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Pro Gln Gly Ile Tyr Asp Gly Glu Thr Leu Thr Val Ser Phe Pro Tyr
            35                  40                  45


Thr Val Ile Gly Asp Pro Ser Gly Thr Thr Val Phe Ser Ala Gly Glu
        50                  55                  60


Leu Thr Leu Lys Asn Leu Asp Asn Ser Ile Ala Ala Leu Pro Leu Ser
 65                  70                  75                  80


Cys Phe Gly Asn Leu Leu Gly Ser Phe Thr Val Leu Gly Arg Gly His
                85                  90                  95


Ser Leu Thr Phe Glu Asn Ile Arg Thr Ser Thr Asn Gly Ala Ala Leu
                100                 105                 110


Ser Asn Ser Ala Ala Asp Gly Leu Phe Thr Ile Glu Gly Phe Lys Glu
            115                 120                 125


Leu Ser Phe Ser Asn Cys Asn Ser Leu Leu Ala Val Leu Pro Ala Ala
        130                 135                 140


Thr Thr Asn Lys Gly Ser Gln Thr Pro Thr Thr Thr Ser Thr Pro Ser
145                 150                 155                 160


Asn Gly Thr Ile Tyr Ser Lys Thr Asp Leu Leu Leu Leu Asn Asn Glu
                165                 170                 175


Lys Phe Ser Phe Tyr Ser Asn Leu Val Ser Gly Asp Gly Gly Ala Ile
            180                 185                 190
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Asp Ala Lys Ser Leu Thr Val Gln Gly Ile Ser Lys Leu Cys Val Phe
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Gln Glu Asn Thr Ala Gln Ala Asp Gly Gly Ala Cys Gln Val Val Thr
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225   230   235   240

Val Ala Gly Val Arg Gly Gly Gly Ile Ala Ala Val Gln Asp Gly Gln
                245   250   255

Gln Gly Val Ser Ser Ser Thr Ser Thr Glu Asp Pro Val Val Ser Phe
                260   265   270

Ser Arg Asn Thr Ala Val Glu Phe Asp Gly Asn Val Ala Arg Val Gly
        275   280   285

Gly Gly Ile Tyr Ser Tyr Gly Asn Val Ala Phe Leu Asn Asn Gly Lys
        290   295   300

Thr Leu Phe Leu Asn Asn Val Ala Ser Pro Val Tyr Ile Ala Ala Lys
305   310   315   320

Gln Pro Thr Ser Gly Gln Ala Ser Asn Thr Ser Asn Asn Tyr Gly Asp
                325   330   335

Gly Gly Ala Ile Phe Cys Lys Asn Gly Ala Gln Ala Gly Ser Asn Asn
                340   345   350

Ser Gly Ser Val Ser Phe Asp Gly Glu Gly Val Val Phe Phe Ser Ser
        355   360   365

Asn Val Ala Ala Gly Lys Gly Gly Ala Ile Tyr Ala Lys Lys Leu Ser
        370   375   380

Val Ala Asn Cys Gly Pro Val Gln Phe Leu Arg Asn Ile Ala Asn Asp
385   390   395   400

Gly Gly Ala Ile Tyr Leu Gly Glu Ser Gly Glu Leu Ser Leu Ser Ala
                405   410   415

Asp Tyr Gly Asp Ile Ile Phe Asp Gly Asn Leu Lys Arg Thr Ala Lys
                420   425   430

Glu Asn Ala Ala Asp Val Asn Gly Val Thr Val Ser Ser Gln Ala Ile
        435   440   445

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Ser Met Gly Ser Gly Gly Lys Ile Thr Thr Leu Arg Ala Lys Ala Gly
    450   455   460

His Gln Ile Leu Phe Asn Asp Pro Ile Glu Met Ala Asn Gly Asn Asn
465   470   475   480

Gln Pro Ala Gln Ser Ser Lys Leu Leu Lys Ile Asn Asp Gly Glu Gly
                485   490   495

Tyr Thr Gly Asp Ile Val Phe Ala Asn Gly Ser Ser Thr Leu Tyr Gln
            500   505   510

Asn Val Thr Ile Glu Gln Gly Arg Ile Val Leu Arg Glu Lys Ala Lys
        515   520   525

Leu Ser Val Asn Ser Leu Ser Gln Thr Gly Gly Ser Leu Tyr Met Glu
    530   535   540

Ala Gly Ser Thr Trp Asp Phe Val Thr Pro Gln Pro Pro Gln Gln Pro
545   550   555   560

Pro Ala Ala Asn Gln Leu Ile Thr Leu Ser Asn Leu His Leu Ser Leu
                565   570   575

Ser Ser Leu Leu Ala Asn Asn Ala Val Thr Asn Pro Pro Thr Asn Pro
                580   585   590

Pro Ala Gln Asp Ser His Pro Ala Val Ile Gly Ser Thr Thr Ala Gly
            595   600   605

Ser Val Thr Ile Ser Gly Pro Ile Phe Phe Glu Asp Leu Asp Asp Thr
    610   615   620

Ala Tyr Asp Arg Tyr Asp Trp Leu Gly Ser Asn Gln Lys Ile Asn Val
625   630   635   640

Leu Lys Leu Gln Leu Gly Thr Lys Pro Pro Ala Asn Ala Pro Ser Asp
                645   650   655

Leu Thr Leu Gly Asn Glu Met Pro Lys Tyr Gly Tyr Gln Gly Ser Trp
            660   665   670

Lys Leu Ala Trp Asp Pro Asn Thr Ala Asn Asn Gly Pro Tyr Thr Leu
        675   680   685

Lys Ala Thr Trp Thr Lys Thr Gly Tyr Asn Pro Gly Pro Glu Arg Val
    690   695   700

Ala Ser Leu Val Pro Asn Ser Leu Trp Gly Ser Ile Leu Asp Ile Arg
705   710   715   720

Ser Ala His Ser Ala Ile Gln Ala Ser Val Asp Gly Arg Ser Tyr Cys
                725   730   735

Arg Gly Leu Trp Val Ser Gly Val Ser Asn Phe Phe Tyr His Asp Arg
            740   745   750

Asp Ala Leu Gly Gln Gly Tyr Arg Tyr Ile Ser Gly Gly Tyr Ser Leu
            755   760   765

Gly Ala Asn Ser Tyr Phe Gly Ser Ser Met Phe Gly Leu Ala Phe Thr
            770   775   780

Glu Val Phe Gly Arg Ser Lys Asp Tyr Val Val Cys Arg Ser Asn His
785   790   795   800

His Ala Cys Ile Gly Ser Val Tyr Leu Ser Thr Gln Gln Ala Leu Cys
                805   810   815

Gly Ser Tyr Leu Phe Gly Asp Ala Phe Ile Arg Ala Ser Tyr Gly Phe
                820   825   830

Gly Asn Gln His Met Lys Thr Ser Tyr Thr Phe Ala Glu Glu Ser Asp
            835   840   845

Val Arg Trp Asp Asn Asn Cys Leu Ala Gly Glu Ile Gly Ala Gly Leu
        850   855   860

Pro Ile Val Ile Thr Pro Ser Lys Leu Tyr Leu Asn Glu Leu Arg Pro
865   870   875   880

Phe Val Gln Ala Glu Phe Ser Tyr Ala Asp His Glu Ser Phe Thr Glu
                885   890   895

Glu Gly Asp Gln Ala Arg Ala Phe Lys Ser Gly His Leu Leu Asn Leu
            900   905   910

Ser Val Pro Val Gly Val Lys Phe Asp Arg Cys Ser Ser Thr His Pro
        915   920   925

Asn Lys Tyr Ser Phe Met Ala Ala Tyr Ile Cys Asp Ala Tyr Arg Thr
        930   935   940

Ile Ser Gly Thr Glu Thr Thr Leu Leu Ser His Gln Glu Thr Trp Thr
945   950   955   960

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Arg Val Arg Phe
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acaagcttta tgtggatcct atttgttcgg agatgcgttt atccgtgcta gctacgggtt 900
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cctatcccat caagagacat ggacaacaga tgcctttcat ttagcaagac atggagttgt 1320
ggttagagga tctatgtatg cttctctaac aagtaatata gaagtatatg ccatggaag 1380
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ctaa                                                              1444
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<210> 12
<211> 56
<212> DNA
<213> Chlamydia
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<400> 12
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<210> 13
<211> 56
<212> DNA
<213> Chlamydia

<400> 13
aagggcccaa ttacgcagag ggtaccctaa gaagaaggca tgccgtgcta gcggag   56

<210> 14
<211> 57
<212> DNA
<213> Chlamydia

<400> 14
aagggcccaa ttacgcagag ggtaccggag agctcgcgaa tccatacgaa taggaac   57

<210> 15
<211> 1013
<212> PRT
<213> Chlamydia

<400> 15
Met Gln Thr Ser Phe His Lys Phe Phe Leu Ser Met Ile Leu Ala Tyr
 1   5   10   15

Ser Cys Cys Ser Leu Asn Gly Gly Gly Tyr Ala Ala Glu Ile Met Val
            20   25   30

Pro Gln Gly Ile Tyr Asp Gly Glu Thr Leu Thr Val Ser Phe Pro Tyr
        35   40   45

Thr Val Ile Gly Asp Pro Ser Gly Thr Thr Val Phe Ser Ala Gly Glu
    50   55   60

Leu Thr Leu Lys Asn Leu Asp Asn Ser Ile Ala Ala Leu Pro Leu Ser
 65   70   75   80

Cys Phe Gly Asn Leu Leu Gly Ser Phe Thr Val Leu Gly Arg Gly His
                85   90   95

Ser Leu Thr Phe Glu Asn Ile Arg Thr Ser Thr Asn Gly Ala Ala Leu
            100   105   110

Ser Asp Ser Ala Asn Ser Gly Leu Phe Thr Ile Glu Gly Phe Lys Glu

```
              115                    120                    125

    Leu Ser Phe Ser Asn Cys Asn Pro Leu Leu Ala Val Leu Pro Ala Ala
         130                    135                    140

    Thr Thr Asn Asn Gly Ser Gln Thr Pro Ser Thr Thr Ser Thr Pro Ser
    145                    150                    155                    160

    Asn Gly Thr Ile Tyr Ser Lys Thr Asp Leu Leu Leu Leu Asn Asn Glu
                        165                    170                    175

    Lys Phe Ser Phe Tyr Ser Asn Ser Val Ser Gly Asp Gly Gly Ala Ile
                   180                    185                    190

    Asp Ala Lys Ser Leu Thr Val Gln Gly Ile Ser Lys Leu Cys Val Phe
                   195                    200                    205

    Gln Glu Asn Thr Ala Gln Ala Asp Gly Gly Ala Cys Gln Val Val Thr
         210                    215                    220

    Ser Phe Ser Ala Met Ala Asn Glu Ala Pro Ile Ala Phe Val Ala Asn
    225                    230                    235                    240

    Val Ala Gly Val Arg Gly Gly Gly Ile Ala Ala Val Gln Asp Gly Gln
                   245                    250                    255

    Gln Gly Val Ser Ser Ser Thr Ser Thr Glu Asp Pro Val Val Ser Phe
                   260                    265                    270

    Ser Arg Asn Thr Ala Val Glu Phe Asp Gly Asn Val Ala Arg Val Gly
              275                    280                    285

    Gly Gly Ile Tyr Ser Tyr Gly Asn Val Ala Phe Leu Asn Asn Gly Lys
         290                    295                    300

    Thr Leu Phe Leu Asn Asn Val Ala Ser Pro Val Tyr Ile Ala Ala Glu
    305                    310                    315                    320

    Gln Pro Thr Asn Gly Gln Ala Ser Asn Thr Ser Asp Asn Tyr Gly Asp
                   325                    330                    335

    Gly Gly Ala Ile Phe Cys Lys Asn Gly Ala Gln Ala Ala Gly Ser Asn
                   340                    345                    350

    Asn Ser Gly Ser Val Ser Phe Asp Gly Glu Gly Val Val Phe Phe Ser
              355                    360                    365

    Ser Asn Val Ala Ala Gly Lys Gly Gly Ala Ile Tyr Ala Lys Lys Leu
```

```
        370                    375                    380
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Ser Val Ala Asn Cys Gly Pro Val Gln Leu Leu Gly Asn Il Ala Asn
385   390   395   400

Asp Gly Gly Ala Ile Tyr Leu Gly Glu Ser Gly Glu Leu Ser Leu Ser
                405   410   415

Ala Asp Tyr Gly Asp Met Ile Phe Asp Gly Asn Leu Lys Arg Thr Ala
            420   425   430

Lys Glu Asn Ala Ala Asp Val Asn Gly Val Thr Val Ser Ser Gln Ala
        435   440   445

Ile Ser Met Gly Ser Gly Gly Lys Ile Thr Thr Leu Arg Ala Lys Ala
        450   455   460

Gly His Gln Ile Leu Phe Asn Asp Pro Ile Glu Met Ala Asn Gly Asn
465   470   475   480

Asn Gln Pro Ala Gln Ser Ser Glu Pro Leu Lys Ile Asn Asp Gly Glu
                485   490   495

Gly Tyr Thr Gly Asp Ile Val Phe Ala Asn Gly Asn Ser Thr Leu Tyr
            500   505   510

Gln Asn Val Thr Ile Glu Gln Gly Arg Ile Val Leu Arg Glu Lys Ala
        515   520   525

Lys Leu Ser Val Asn Ser Leu Ser Gln Thr Gly Gly Ser Leu Tyr Met
        530   535   540

Glu Ala Gly Ser Thr Leu Asp Phe Val Thr Pro Gln Pro Pro Gln Gln
545   550   555   560

Pro Pro Ala Ala Asn Gln Ser Ile Thr Leu Ser Asn Leu His Leu Ser
                565   570   575

Leu Ser Ser Leu Leu Ala Asn Asn Ala Val Thr Asn Pro Pro Thr Asn
        580   585   590

Pro Pro Ala Gln Asp Ser His Pro Ala Val Ile Gly Ser Thr Thr Ala
        595   600   605

Gly Ser Val Thr Ile Ser Gly Pro Ile Phe Phe Glu Asp Leu Asp Asp
    610   615   620

Thr Ala Tyr Asp Arg Tyr Asp Trp Leu Gly Ser Asn Gln Lys Ile Asp

<pre>
625   630   635   640

Val Leu Lys Leu Gln Leu Gly Thr Gln Pro Pro Ala Asn Ala Pro Ser
            645   650   655

Asp Leu Thr Leu Gly Asn Glu Met Pro Lys Tyr Gly Tyr Gln Gly Ser
            660   665   670

Trp Lys Leu Ala Trp Asp Pro Asn Thr Ala Asn Asn Gly Pro Tyr Thr
            675   680   685

Leu Lys Ala Thr Trp Thr Lys Thr Gly Tyr Asn Pro Gly Pro Glu Arg
        690   695   700

Val Ala Ser Leu Val Pro Asn Ser Leu Trp Gly Ser Ile Leu Asp Ile
705   710   715   720

Arg Ser Ala His Ser Ala Ile Gln Ala Ser Val Asp Gly Arg Ser Tyr
            725   730   735

Cys Arg Gly Leu Trp Val Ser Gly Val Ser Asn Phe Phe Tyr His Asp
            740   745   750

Arg Asp Ala Leu Gly Gln Gly Tyr Arg Tyr Ile Ser Gly Gly Tyr Ser
            755   760   765

Leu Gly Ala Asn Ser Tyr Phe Gly Ser Ser Met Phe Gly Leu Ala Phe
        770   775   780

Thr Glu Val Phe Gly Arg Ser Lys Asp Tyr Val Val Cys Arg Ser Asn
785   790   795   800

His His Ala Cys Ile Gly Ser Val Tyr Leu Ser Thr Lys Gln Ala Leu
                805   810   815

Cys Gly Ser Tyr Val Phe Gly Asp Ala Phe Ile Arg Ala Ser Tyr Gly
            820   825   830

Phe Gly Asn Gln His Met Lys Thr Ser Tyr Thr Phe Ala Glu Glu Ser
            835   840   845

Asp Val Cys Trp Asp Asn Asn Cys Leu Val Gly Glu Ile Gly Val Gly
        850   855   860

Leu Pro Ile Val Ile Thr Pro Ser Lys Leu Tyr Leu Asn Glu Leu Arg
865   870   875   880

Pro Phe Val Gln Ala Glu Phe Ser Tyr Ala Asp His Glu Ser Phe Thr
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                    885                    890                    895

Glu Glu Gly Asp Gln Ala Arg Ala Phe Arg Ser Gly His Leu Met Asn
            900                    905                    910

Leu Ser Val Pro Val Gly Val Lys Phe Asp Arg Cys Ser Ser Thr His
        915                    920                    925

Pro Asn Lys Tyr Ser Phe Met Gly Ala Tyr Ile Cys Asp Ala Tyr Arg
        930                    935                    940

Thr Ile Ser Gly Thr Gln Thr Thr Leu Leu Ser His Gln Glu Thr Trp
945                    950                    955                    960

Thr Thr Asp Ala Phe His Leu Ala Arg His Gly Val Ile Val Arg Gly
                965                    970                    975

Ser Met Tyr Ala Ser Leu Thr Ser Asn Ile Glu Val Tyr Gly His Gly
        980                    985                    990

Arg Tyr Glu Tyr Arg Asp Thr Ser Arg Gly Tyr Gly Leu Ser Ala Gly
        995                    1000                    1005

Ser Lys Val Arg Phe
    1010
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<210> 16
<211> 1013
<212> PRT
<213> Chlamydia

<400> 16
Met Gln Thr Ser Phe His Lys Phe Phe Leu Ser Met Ile Leu Ala Tyr
  1               5                   10                  15

Ser Cys Cys Ser Leu Thr Gly Gly Gly Tyr Ala Ala Glu Ile Met Val
            20                  25                  30

Pro Gln Gly Ile Tyr Asp Gly Glu Thr Leu Thr Val Ser Phe Pro Tyr
        35                  40                  45

Thr Val Ile Gly Asp Pro Ser Gly Thr Thr Val Phe Ser Ala Gly Glu
        50                  55                  60

Leu Thr Leu Lys Asn Leu Asp Asn Ser Ile Ala Ala Leu Pro Leu Ser
65                  70                  75                  80
```

Cys Phe Gly Asn Leu Leu Gly Ser Phe Thr Val Leu Gly Arg Gly His
                85   90   95

Ser Leu Thr Phe Glu Asn Ile Arg Thr Ser Thr Asn Gly Ala Ala Leu
            100   105   110

Ser Asp Ser Ala Asn Ser Gly Leu Phe Thr Ile Glu Gly Phe Lys Glu
            115   120   125

Leu Ser Phe Ser Asn Cys Asn Ser Leu Leu Ala Val Leu Pro Ala Ala
        130   135   140

Thr Thr Asn Asn Gly Ser Gln Thr Pro Thr Thr Thr Ser Thr Pro Ser
145   150   155   160

Asn Gly Thr Ile Tyr Ser Lys Thr Asp Leu Leu Leu Leu Asn Asn Glu
                165   170   175

Lys Phe Ser Phe Tyr Ser Asn Leu Val Ser Gly Asp Gly Gly Thr Ile
            180   185   190

Asp Ala Lys Ser Leu Thr Val Gln Gly Ile Ser Lys Leu Cys Val Phe
            195   200   205

Gln Glu Asn Thr Ala Gln Ala Asp Gly Gly Ala Cys Gln Val Val Thr
    210   215   220

Ser Phe Ser Ala Met Ala Asn Glu Ala Pro Ile Ala Phe Ile Ala Asn
225   230   235   240

Val Ala Gly Val Arg Gly Gly Gly Ile Ala Ala Val Gln Asp Gly Gln
                245   250   255

Gln Gly Val Ser Ser Ser Thr Ser Thr Glu Asp Pro Val Val Ser Phe
            260   265   270

Ser Arg Asn Thr Ala Val Glu Phe Asp Gly Asn Val Ala Arg Val Gly
        275   280   285

Gly Gly Ile Tyr Ser Tyr Gly Asn Val Ala Phe Leu Asn Asn Gly Lys
    290   295   300

Thr Leu Phe Leu Asn Asn Val Ala Ser Pro Val Tyr Ile Ala Ala Glu
305   310   315   320

Gln Pro Thr Asn Gly Gln Ala Ser Asn Thr Ser Asp Asn Tyr Gly Asp
            325   330   335

Gly Gly Ala Ile Phe Cys Lys Asn Gly Ala Gln Ala Ala Gly Ser Asn
          340   345   350

Asn Ser Gly Ser Val Ser Phe Asp Gly Glu Gly Val Val Phe Phe Ser
          355   360   365

Ser Asn Val Ala Ala Gly Lys Gly Gly Ala Ile Tyr Ala Lys Lys Leu
    370   375   380

Ser Val Ala Asn Cys Gly Pro Val Gln Phe Leu Gly Asn Ile Ala Asn
385   390   395   400

Asp Gly Gly Ala Ile Tyr Leu Gly Glu Ser Gly Glu Leu Ser Leu Ser
              405   410   415

Ala Asp Tyr Gly Asp Ile Ile Phe Asp Gly Asn Leu Lys Arg Thr Ala
          420   425   430

Lys Glu Asn Ala Ala Asp Val Asn Gly Val Thr Val Ser Ser Gln Ala
          435   440   445

Ile Ser Met Gly Ser Gly Gly Lys Ile Thr Thr Leu Arg Ala Lys Ala
    450   455   460

Gly His Gln Ile Leu Phe Asn Asp Pro Ile Glu Met Ala Asn Gly Asn
465   470   475   480

Asn Gln Pro Ala Gln Ser Ser Glu Pro Leu Lys Ile Asn Asp Gly Glu
              485   490   495

Gly Tyr Thr Gly Asp Ile Val Phe Ala Asn Gly Asn Ser Thr Leu Tyr
          500   505   510

Gln Asn Val Thr Ile Glu Gln Gly Arg Ile Val Leu Arg Glu Lys Ala
          515   520   525

Lys Leu Ser Val Asn Ser Leu Ser Gln Thr Gly Gly Ser Leu Tyr Met
    530   535   540

Glu Ala Gly Ser Thr Leu Asp Phe Val Thr Pro Gln Pro Gln Gln
545   550   555   560

Pro Pro Ala Ala Asn Gln Leu Ile Thr Leu Ser Asn Leu His Leu Ser
              565   570   575

Leu Ser Ser Leu Leu Ala Asn Asn Ala Val Thr Asn Pro Pro Thr Asn
          580   585   590

- 16 -

Pro Pro Ala Gln Asp Ser His Pro Ala Val Ile Gly Ser Thr Thr Ala
        595   600   605

Gly Pro Val Thr Ile Ser Gly Pro Phe Phe Phe Glu Asp Leu Asp Asp
        610   615   620

Thr Ala Tyr Asp Arg Tyr Asp Trp Leu Gly Ser Asn Gln Lys Ile Asp
625   630   635   640

Val Leu Lys Leu Gln Leu Gly Thr Gln Pro Ser Ala Asn Ala Pro Ser
        645   650   655

Asp Leu Thr Leu Gly Asn Glu Met Pro Lys Tyr Gly Tyr Gln Gly Ser
        660   665   670

Trp Lys Leu Ala Trp Asp Pro Asn Thr Ala Asn Asn Gly Pro Tyr Thr
        675   680   685

Leu Lys Ala Thr Trp Thr Lys Thr Gly Tyr Asn Pro Gly Pro Glu Arg
        690   695   700

Val Ala Ser Leu Val Pro Asn Ser Leu Trp Gly Ser Ile Leu Asp Ile
705   710   715   720

Arg Ser Ala His Ser Ala Ile Gln Ala Ser Val Asp Gly Arg Ser Tyr
        725   730   735

Cys Arg Gly Leu Trp Val Ser Gly Val Ser Asn Phe Ser Tyr His Asp
        740   745   750

Arg Asp Ala Leu Gly Gln Gly Tyr Arg Tyr Ile Ser Gly Gly Tyr Ser
        755   760   765

Leu Gly Ala Asn Ser Tyr Phe Gly Ser Ser Met Phe Gly Leu Ala Phe
        770   775   780

Thr Glu Val Phe Gly Arg Ser Lys Asp Tyr Val Val Cys Arg Ser Asn
785   790   795   800

His His Ala Cys Ile Gly Ser Val Tyr Leu Ser Thr Lys Gln Ala Leu
                805   810   815

Cys Gly Ser Tyr Leu Phe Gly Asp Ala Phe Ile Arg Ala Ser Tyr Gly
                820   825   830

Phe Gly Asn Gln His Met Lys Thr Ser Tyr Thr Phe Ala Glu Glu Ser
        835   840   845

Asp Val Arg Trp Asp Asn Asn Cys Leu Val Gly Glu Ile Gly Val Gly
    850   855   860

Leu Pro Ile Val Thr Thr Pro Ser Lys Leu Tyr Leu Asn Glu Leu Arg
865   870   875   880

Pro Phe Val Gln Ala Glu Phe Ser Tyr Ala Asp His Glu Ser Phe Thr
            885   890   895

Glu Glu Gly Asp Gln Ala Arg Ala Phe Arg Ser Gly His Leu Met Asn
            900   905   910

Leu Ser Val Pro Val Gly Val Lys Phe Asp Arg Cys Ser Ser Thr His
    915   920   925

Pro Asn Lys Tyr Ser Phe Met Gly Ala Tyr Ile Cys Asp Ala Tyr Arg
    930   935   940

Thr Ile Ser Gly Thr Gln Thr Thr Leu Leu Ser His Gln Glu Thr Trp
945   950   955   960

Thr Thr Asp Ala Phe His Leu Ala Arg His Gly Val Ile Val Arg Gly
            965   970   975

Ser Met Tyr Ala Ser Leu Thr Ser Asn Ile Glu Val Tyr Gly His Gly
            980   985   990

Arg Tyr Glu Tyr Arg Asp Thr Ser Arg Gly Tyr Gly Leu Ser Ala Gly
    995   1000   1005

Ser Lys Val Arg Phe
    1010

<210> 17
<211> 505
<212> PRT
<213> Chlamydia

<400> 17
Glu Ile Met Val Pro Gln Gly Ile Tyr Asp Gly Glu Thr Leu Thr Val
1   5   10   15

Ser Phe Pro Tyr Thr Val Ile Gly Asp Pro Ser Gly Thr Thr Val Phe
            20   25   30

Ser Ala Gly Glu Leu Thr Leu Lys Asn Leu Asp Asn Ser Ile Ala Ala
        35   40   45

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Leu Pro Leu Ser Cys Phe Gly Asn Leu Leu Gly Ser Phe Thr Val Leu
    50                  55                  60

Gly Arg Gly His Ser Leu Thr Phe Glu Asn Ile Arg Thr Ser Thr Asn
65                  70                  75                  80

Gly Ala Ala Leu Ser Asn Ser Ala Ala Asp Gly Leu Phe Thr Ile Glu
                85                  90                  95

Gly Phe Lys Glu Leu Ser Phe Ser Asn Cys Asn Ser Leu Leu Ala Val
            100                 105                 110

Leu Pro Ala Ala Thr Thr Asn Lys Gly Ser Gln Thr Pro Thr Thr Thr
            115                 120                 125

Ser Thr Pro Ser Asn Gly Thr Ile Tyr Ser Lys Thr Asp Leu Leu Leu
    130                 135                 140

Leu Asn Asn Glu Lys Phe Ser Phe Tyr Ser Asn Leu Val Ser Gly Asp
145                 150                 155                 160

Gly Gly Ala Ile Asp Ala Lys Ser Leu Thr Val Gln Gly Ile Ser Lys
                165                 170                 175

Leu Cys Val Phe Gln Glu Asn Thr Ala Gln Ala Asp Gly Gly Ala Cys
            180                 185                 190

Gln Val Val Thr Ser Phe Ser Ala Met Ala Asn Glu Ala Pro Ile Ala
        195                 200                 205

Phe Val Ala Asn Val Ala Gly Val Arg Gly Gly Gly Ile Ala Ala Val
    210                 215                 220

Gln Asp Gly Gln Gln Gly Val Ser Ser Ser Thr Ser Thr Glu Asp Pro
225                 230                 235                 240

Val Val Ser Phe Ser Arg Asn Thr Ala Val Glu Phe Asp Gly Asn Val
            245                 250                 255

Ala Arg Val Gly Gly Gly Ile Tyr Ser Tyr Gly Asn Val Ala Phe Leu
            260                 265                 270

Asn Asn Gly Lys Thr Leu Phe Leu Asn Asn Val Ala Ser Pro Val Tyr
        275                 280                 285

Ile Ala Ala Lys Gln Pro Thr Ser Gly Gln Ala Ser Asn Thr Ser Asn
    290                 295                 300
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Asn Tyr Gly Asp Gly Gly Ala Ile Phe Cys Lys Asn Gly Ala Gln Ala
305             310             315             320

Gly Ser Asn Asn Ser Gly Ser Val Ser Phe Asp Gly Glu Gly Val Val
            325             330             335

Phe Phe Ser Ser Asn Val Ala Ala Gly Lys Gly Gly Ala Ile Tyr Ala
        340             345             350

Lys Lys Leu Ser Val Ala Asn Cys Gly Pro Val Gln Phe Leu Arg Asn
        355             360             365

Ile Ala Asn Asp Gly Gly Ala Ile Tyr Leu Gly Glu Ser Gly Glu Leu
    370             375             380

Ser Leu Ser Ala Asp Tyr Gly Asp Ile Ile Phe Asp Gly Asn Leu Lys
385             390             395             400

Arg Thr Ala Lys Glu Asn Ala Ala Asp Val Asn Gly Val Thr Val Ser
            405             410             415

Ser Gln Ala Ile Ser Met Gly Ser Gly Gly Lys Ile Thr Thr Leu Arg
            420             425             430

Ala Lys Ala Gly His Gln Ile Leu Phe Asn Asp Pro Ile Glu Met Ala
        435             440             445

Asn Gly Asn Asn Gln Pro Ala Gln Ser Ser Lys Leu Leu Lys Ile Asn
    450             455             460

Asp Gly Glu Gly Tyr Thr Gly Asp Ile Val Phe Ala Asn Gly Ser Ser
465             470             475             480

Thr Leu Tyr Gln Asn Val Thr Ile Glu Gln Gly Arg Ile Val Leu Arg
            485             490             495

Glu Lys Ala Lys Leu Ser Val Asp Ser
        500             505
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<210> 18
<211> 57
<212> DNA
<213> Chlamydia

<400> 18
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<210> 19
<211> 20
<212> DNA
<213> Chlamydia

<400> 19
cgctctagaa ctagtggatc   20

<210> 20
<211> 22
<212> DNA
<213> Chlamydia

<400> 20
atggttcctc aaggaattta cg   22

<210> 21
<211> 19
<212> DNA
<213> Chlamydia

<400> 21
ggtcccccat cagcgggag   19

<210> 22
<211> 1515
<212> DNA
<213> Chlamydia

<400> 22
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aatcttgaca attctattgc agctttgcct ttaagttgtt ttgggaactt attaggagt 180
tttactgttt tagggagagg acactcgttg actttcgaga acatacggac ttctacaaat 240
ggggcagctc taagtaatag cgctgctgat ggactgttta ctattgaggg ttttaaagaa 300
ttatcctttt ccaattgcaa ttcattactt gccgtactgc ctgctgcaac gactaataag 360
ggtagccaga ctccgacgac aacatctaca ccgtctaatg gtactattta ttctaaaaca 420
gatcttttgt tactcaataa tgagaagttc tcattctata gtaatttagt ctctggagat 480
gggggagcta tagatgctaa gagcttaacg gttcaaggaa ttagcaagct ttgtgtcttc 540
caagaaaata ctgctcaagc tgatggggga gcttgtcaag tagtcaccag tttctctgct 600
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agaacagcca aagagaatgc tgccgatgtt aatggcgtaa ctgtgtcctc acaagccatt 1260
tcgatgggat cgggagggaa aataacgaca ttaagagcta aagcagggca tcagattctc 1320
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actttgtacc aaaatgttac gatagagcaa ggaaggattg ttcttcgtga aaaggcaaaa 1500
ttatcagtga attct                                                    1515
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<210> 23
<211> 3354
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
      recombinant expression vector

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ttaactgtat catttcccta tactgttata ggagatccga gtgggactac tgttttttct 180
gcaggagagt taacgttaaa aaatcttgac aattctattg cagctttgcc tttaagttgt 240
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aacatacgga cttctacaaa tggagctgca ctaagtgaca gcgctaatag cgggttattt 360
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gtaactgtgt cctcacaagc catttcgatg ggatcgggag ggaaaataac gacattaaga 1380
gctaaagcag ggcatcagat tctctttaat gatcccatcg agatggcaaa cggaaataac 1440
cagccagcgc agtcttccga acctctaaaa attaacgatg tgaaggata cacaggggat 1500
attgtttttg ctaatggaaa cagtactttg taccaaaatg ttacgataga gcaaggaagg 1560
attgttcttc gtgaaaaggc aaaattatca gtgaattctc taagtcagac aggtgggagt 1620
ctgtatatgg aagctgggag tacattggat tttgtaactc cacaaccacc acaacagcct 1680
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cctgccgcta atcagtcgat cacgctttcc aatctgcatt tgtctctttc ttctttgtta 1740
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ctgaaattac agttagggac tcagccccca gctaatgccc catcagattt gactctaggg 1980
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Ser Cys Phe Gly Asn Leu Leu Gly Ser Phe Thr Val Leu Gly Arg Gly
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<211> 24
<212> PRT
<213> Chlamydia

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<210> 27
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<212> PRT
<213> Chlamydia

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<210> 28
<211> 7
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<213> Chlamydia

<400> 28
Tyr Gly Asp Ile Ile Phe Asp
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<210> 29
<211> 63
<212> PRT
<213> Chlamydera cerviniventris

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            20   25   30

Thr Thr Val Phe Ser Ala Gly Glu Leu Thr Leu Lys Asn Leu Asp Asn
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            20   25   30

Gly Ser Asn Gln Lys Ile Asn Val Leu Lys Leu Gln Leu Gly Thr Lys
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<210> 36
<211> 458
<212> PRT
<213> Chlamydia

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Ala Pro Ile Ala Phe Val Ala Asn Val Ala Gly Val Arg Gly Gly Gly
            20                  25                  30

Ile Ala Ala Val Gln Asp Gly Gln Gln Gly Val Ser Ser Ser Thr Ser
            35                  40                  45

Thr Glu Asp Pro Val Val Ser Phe Ser Arg Asn Thr Ala Val Glu Phe
        50                  55                  60

Asp Gly Asn Val Ala Arg Val Gly Gly Gly Ile Tyr Ser Tyr Gly Asn
65                  70                  75                  80

Val Ala Phe Leu Asn Asn Gly Lys Thr Leu Phe Leu Asn Asn Val Ala
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Ser Pro Val Tyr Ile Ala Ala Lys Gln Pro Thr Ser Gly Gln Ala Ser
            100                 105                 110

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Gly Ala Gln Ala Gly Ser Asn Asn Ser Gly Ser Val Ser Phe Asp Gly
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Glu Gly Val Val Phe Phe Ser Ser Asn Val Ala Ala Gly Lys Gly Gly
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    Gly Asn Leu Lys Arg Thr Ala Lys Glu Asn Ala Ala Asp Val Asn Gly
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    Val Thr Val Ser Ser Gln Ala Ile Ser Met Gly Ser Gly Gly Lys Ile
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    Thr Thr Leu Arg Ala Lys Ala Gly His Gln Ile Leu Phe Asn Asp Pro
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    Asn Gly Ser Ser Thr Leu Tyr Gln Asn Val Thr Ile Glu Gln Gly Arg
        290   295   300

    Ile Val Leu Arg Glu Lys Ala Lys Leu Ser Val Asn Ser Leu Ser Gln
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    Thr Gly Gly Ser Leu Tyr Met Glu Ala Gly Ser Thr Trp Asp Phe Val
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    Leu Ser Asn Leu His Leu Ser Leu Ser Ser Leu Leu Ala Asn Asn Ala
                355   360   365

    Val Thr Asn Pro Pro Thr Asn Pro Pro Ala Gln Asp Ser His Pro Ala
        370   375   380

    Val Ile Gly Ser Thr Thr Ala Gly Ser Val Thr Ile Ser Gly Pro Ile
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    Phe Phe Glu Asp Leu Asp Asp Thr Ala Tyr Asp Arg Tyr Asp Trp Leu
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    Gly Ser Asn Gln Lys Ile Asn Val Leu Lys Leu Gln Leu Gly Thr Lys

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Arg Ser Ala His Ser Ala Ile Gln Ala Ser Val Asp Gly Arg Ser Tyr
        35                    40                    45

Cys Arg Gly Leu Trp Val Ser Gly Val Ser Asn Phe Phe Tyr His Asp
    50                    55                    60

Arg Asp Ala Leu Gly Gln Gly Tyr Arg Tyr Ile Ser Gly Gly Tyr Ser
 65                    70                    75                    80

Leu Gly Ala Asn Ser Tyr Phe Gly Ser Ser Met Phe Gly Leu Ala Phe
                85                    90                    95

Thr Glu Val Phe Gly Arg Ser Lys Asp Tyr Val Val Cys Arg Ser Asn
            100                   105                   110

His His Ala Cys Ile Gly Ser Val Tyr Leu Ser Thr Gln Gln Ala Leu
        115                   120                   125

Cys Gly Ser Tyr Leu Phe Gly Asp Ala Phe Ile Arg Ala Ser Tyr Gly
    130                   135                   140

Phe Gly Asn Gln His Met Lys Thr Ser Tyr Thr Phe Ala Glu Glu Ser
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Asp Val Arg Trp Asp Asn Asn Cys Leu Ala Gly Glu Ile Gly Ala Gly
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Glu Glu Gly Asp Gln Ala Arg Ala Phe Lys Ser Gly His Leu Leu Asn
    210                 215                 220

Leu Ser Val Pro Val Gly Val Lys Phe Asp Arg Cys Ser Ser Thr His
225                 230                 235                 240

Pro Asn Lys Tyr Ser Phe Met Ala Ala Tyr Ile Cys Asp Ala Tyr Arg
            245                 250                 255

Thr Ile Ser Gly Thr Glu Thr Thr Leu Leu Ser His Gln Glu Thr Trp
            260                 265                 270

Thr Thr Asp Ala Phe His Leu Ala Arg His Gly Val Val Val Arg Gly
            275                 280                 285

Ser Met Tyr Ala Ser Leu Thr Ser Asn Ile Glu Val Tyr Gly His Gly
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<223> Description of Artificial Sequence: primer

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/20737

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, SCISEARCH, EMBASE, BIOSIS, CAPLUS, BIOTECHDS, CONFSCI, LIFESCI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,427,782 A (CALDWELL et al.) 24 January 1984, column 3, line 13 to column 7, line 68.	1-6, 16-17, 19-24, 26-39
X	US 5,071,962 A (MORRISON et al.) 10 December 1991, column 4, line 10 to column 8, line 51.	1-24, 26-41
X	US 5,516,638 A (URNOVITZ et al.) 14 May 1996, column 2, line 6 to column 6, line 24.	23-24, 26-30, 32-34
X,P	US 5,725,863 A (DANIELS et al.) 10 March 1998, column 3, line 65 to column 4, line 47.	1-6, 16-17, 19 - 24, 26-39

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01 MARCH 1999	**24 MAR 1999**

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	STEPHEN GUCKER
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/20737

Box I   Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 25
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II   Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

   Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest   ☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1))(July 1992)*

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 9/14, 9/48, 9/127, 38/16, 39/00, 39/02, 39/118, 39/395, 39/40; CO7H 21/04; CO7K 14/00, 14/195, 14/295, 16/00, 16/12; C12N 5/00, 15/63; C12Q 1/68; GO1N 33/53, 33/563, 33/571

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/139.1, 164.1, 184.1, 185.1, 190.1, 263.1, 450, 451, 489; 435/6, 7.36, 320.1, 325, 975; 436/513; 514/2, 12, 824, 931, 962; 530/300, 350, 387.1, 389.1, 389.5; 536/23.7

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

424/139.1, 164.1, 184.1, 185.1, 190.1, 263.1, 450, 451, 489; 435/6, 7.36, 320.1, 325, 975; 436/513; 514/2, 12, 824, 931, 962; 530/300, 350, 387.1, 389.1, 389.5; 536/23.7

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-6, 16-17, 19-20, 25, 30-31, and 35-39, drawn to protein or protein compositions.

Group II, claims 7-15 and 18, drawn to nucleic acid, vectors, and host cells.

Group III, claims 21-22, drawn to a method of producing an immune response.

Group IV, claims 23-24, drawn to antisera and antibodies.

Group V, claims 26-27, drawn to methods of detecting antibodies and a kit.

Group VI, claims 28-29, drawn to methods of detecting Chlamydia and a kit.

Group VII, claims 32-34, drawn to methods of treatment.

Group VIII, claims 40-41, drawn to methods of detecting nucleic acid and a kit.

The inventions listed as Groups I-VIII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking Groups I-VIII appears to be that they all relate to a Chlamydia protein, fragment, or analogue thereof. However, Caldwell et al. (US 4,427,782, 1984) teach isolation of principal outer membrane protein and antigen of Chlamydia which appears to be encompassed by the scope of claim 1 ("fragment or analogue thereof"). Therefore, the technical feature linking the inventions of Groups I-VIII does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of Group I is considered to be a HMW protein from Chlamydia.

The special technical feature of Group II is considered to be a nucleic acid encoding a HMW protein from Chlamydia.

The special technical feature of Group III is considered to be a method of producing an immune response against a HMW protein from Chlamydia.

The special technical feature of Group IV is considered to be antibodies against a HMW protein from Chlamydia.

The special technical feature of Group V is considered to be methods of detecting antibodies against a HMW protein

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/20737

from Chlamydia.

The special technical feature of Group VI is considered to be methods of detecting Chlamydia by detecting a HMW protein from Chlamydia.

The special technical feature of Group VII is considered to be methods of treating Chlamydia infection by using a HMW protein from Chlamydia.

. The special technical feature of Group VIII is considered to be methods of detecting Chlamydia by detecting a nucleic acid encoding a HMW protein from Chlamydia.

Accordingly, Groups I-VIII are not so linked by the same or a corresponding special technical feature as to form a single general inventive concept.

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